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ERRATA AND AUTHORS' EMENDATIONS

Page 60, Table XIII, heading "*Folyporus dryophilus*" should read "*Polyporus dryophilus*."
Page 105, Table I, "Peach (*Prunus persica*)" should read "Peach (*Amygdalus persica*)."
Page 321, line 8, "clay" should read "clay loam."
Page 335, line 5 from bottom, "(fig. 3–6)" should read "(fig. 8–20)."
Page 361, line 18 from bottom, omit "10."
Page 366, citation 27, "1917" should read "1918."
Pages 364–368, in citations 2, 16, 29, 30, 56, and 57, omit "Not seen,"
Pages 394–395, citations 3 and 8, "1917" should read "1918."
Page 429, line 19 from bottom, "or against tapeworms" should read "and against tapeworms."
Page 430, line 6, "instability" should read "irritability."
Page 455, line 5, "stroke" should read "streak."

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EFFECT OF TIME OF DIGESTION ON THE HYDROLYSIS OF CASEIN IN THE PRESENCE OF STARCH[1]

By J. S. McHARGUE,

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The Van Slyke[2] method for protein analysis was worked out upon mixtures of relatively pure amino acids and was not intended to be applied directly to crude sources of protein contained in cereals and feeding stuffs.

Notwithstanding this fact, Grindley, Slater, et al.,[3] of the Illinois Experiment Station, published in 1915 the results of the determination of the amino acids contained in cottonseed meal, tankage, and alfalfa hay, applying the Van Slyke method directly to the proteins contained in these different feeds.

In the same month of 1915 Nollau,[4] of this Station, published his results, obtained by the Van Slyke method, on about 25 different sources of crude protein contained in various seeds and feeding stuffs.

In December, 1915, Grindley, Slater, et al.,[3] published a second paper on the amino-acid content of various feeds, including wheat, oats, barley, and sov beans, a number of which had been analyzed by Nollau. The summary of their second paper in part is as follows:

> The results here reported confirm the conclusion previously drawn, namely, that the Van Slyke method for the determination of the chemical groups characteristic of the amino acids of proteins can be applied directly to the quantitative determinations of the amino acids of feeding stuffs with at least a fair degree of accuracy.
>
> The results which we have obtained for the quantitative determinations of amino acids in feeding stuffs, on the whole, do not agree well with those recently published by Nollau. In some determinations the results from the two sources are quite satisfactory, but in many cases the agreement is far from satisfactory. The lack of con-

[1] Approved for publication in the Journal of Agricultural Research by A. M. Peter, Acting Director, Kentucky Agricultural Experiment Station.

[2] VAN SLYKE, D. D. THE ANALYSIS OF PROTEINS BY DETERMINATION OF THE CHEMICAL GROUPS CHARACTERISTIC OF THE DIFFERENT AMINO ACIDS. *In* Jour. Biol. Chem., v. 10, no. 1, p. 15–55, 2 fig. 1911.

[3] GRINDLEY, H. S., SLATER, M. E., et al. THE QUANTITATIVE DETERMINATION OF THE AMINO ACIDS OF FEEDING STUFFS BY THE VAN SLYKE METHOD. *In* Jour. Amer. Chem. Soc., v. 37, no. 7, p. 1778–1781; no. 12, p. 2762–2769. 1915.

[4] NOLLAU, E. H. THE AMINO-ACID CONTENT OF CERTAIN COMMERCIAL FEEDING STUFFS AND OTHER SOURCES OF PROTEIN. *In* Jour. Biol. Chem., v. 21, no. 3, p. 611–614. 1915.

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cordant results is probably due in the main to differences in the details of procedure in the experimental work.

Hart and Bentley,[1] of the Wisconsin Experiment Station, comment unfavorably on the lack of agreement between the results obtained by Grindley, Slater, et al., and those obtained by Nollau for the amount of the different amino-acid groups contained in feeding stuffs. They state that whether accurate determinations of any or all the amino acids can be secured when the hydrolyzing proteins are in contact with hydrolyzing carbohydrates must first be determined before these data can be accepted as final.

Presumably in order to substantiate the theory in regard to the effect of hydrolyzing carbohydrates on the different amino-acid groups in proteins, Hart and Sure [2] have published results obtained upon the hydrolysis of casein, alone and in the presence of a number of different carbohydrates. In one of their experiments, 2.4 gm. of casein and 12 gm. of starch were hydrolyzed by boiling in 20 per cent hydrochloric acid for a period of 48 hours. The result obtained for lysin in this experiment shows that approximately 50 per cent of this amino-acid group has been changed to some other form of combination. They summarize their results in part as follows:

> The Van Slyke method of protein analysis, applied to casein, hydrolyzed in the presence of various carbohydrates, brings about a total redistribution of the amino-acids varying with the nature of the carbohydrate employed. This work on casein and Gortner's work on fibrin, hydrolyzed in the presence of cellulose, *definitely* show the inapplicability of the method of direct hydrolysis for the estimation of amino-acids in feeding stuffs by Van Slyke's method. The results so secured will be inaccurate.

Upon the publication of Hart and Sure's results, it appeared to the writer that their conclusions were much broader than their experiments justified. In fact, Hart and Bentley [3] make statements which appear to be merely forecastings rather than conclusions arrived at by experimentation. In order to be able to say positively that the Van Slyke method for protein analysis can not be applied directly to heterogeneous mixtures of protein and carbohydrate requires much further experimentation. It is by no means to be taken for granted that results obtained on a 48-hour digestion will be the same as those carried on for a shorter length of time.

It therefore occurred to the writer that a duplication of the experiment of Hart and Sure upon the effect produced on the hydrolysis of casein by the presence of starch, in which the time of digestion varied, would afford more conclusive evidence on this subject. Accordingly, five experiments were planned, as follows:

[1] HART, E. B., and BENTLEY, W. H. THE CHARACTER OF THE WATER-SOLUBLE NITROGEN OF SOME COMMON FEEDING STUFFS. *In* Jour. Biol. Chem., v. 22, no. 3, p. 477–483. 1915.

[2] ...... and SURE, Barnett. THE INFLUENCE OF CARBOHYDRATES ON THE ACCURACY OF THE VAN SLYKE METHOD IN THE HYDROLYSIS OF CASEIN. *In* Jour. Biol. Chem., v. 28, no. 1, p. 241–249. 1916.

[3] HART, E. B., and BENTLEY, W. H. Op. cit.

Five 10-gm. portions of Hammarsten's casein were weighed out and transferred to five 1-liter round-bottom Jena flasks. Fifty gm. of cornstarch were then weighed out and added to each of the flasks except the first, which contained casein alone. Three hundred c. c. of 20 per cent hydrochloric acid, specific gravity 1.11, were added to each flask. All the flasks were then heated on the water bath, with frequent shakings, for about two hours. The object of this preliminary heating on the water bath was to liquefy the starch-casein mixtures, which had gelatinized upon the addition of the hydrochloric acid. After the starch had become liquid all the flasks were removed and attached to reflux condensers and heated to a gentle boil.

Experiments 1 and 2 were allowed to digest for 12 hours, No. 3 for 15 hours, No. 4 for 24 hours, and No. 5 for 48 hours, each being cut out at the expiration of its time interval.

After each of the experiments had stood at room temperature for six or eight hours, they were filtered through paper on a Buchner funnel and washed practically free of chlorids with hot water. There was no insoluble residue remaining on the filter from the casein digestion. There were rather large insoluble carbonaceous residues remaining from each of the casein-starch mixtures. Each of these was dried at 100° C., bottled, and set aside for further investigation as to their nitrogen content.

The filtrates in each of the experiments were concentrated separately under reduced pressure until practically all of the excess of hydrochloric acid was removed. The residues were taken up in water and run through filters into separate flasks of 250-c. c. capacity. After the filters were washed thoroughly, the contents of each flask were brought up to the mark with water, and duplicate analyses were carried out by the Van Slyke method on aliquots from each of these hydrolyzed solutions. The results obtained are shown in Table I.

From the data in Table I showing the average results obtained upon casein alone and upon definite mixtures of starch and casein digested at different intervals of time the following observations may be made.

In all of the experiments there is but slight variation in the ammonia determinations[1]; the maximum result is obtained in the 48-hour digestion. The increase in this case is in all probability owing to the change of some of the amino groups to ammonia compounds, which indicates overdigestion.

The results for the humin determinations show a diminution in the 15-, 24-, and 48-hour digestions over those of the 12-hour digestions. However, the humin determination in the 12-hour digestion of the starch-casein mixture agrees well with the humin results obtained on casein alone.

[1] Previous to the ammonia determinations the acidity of the hydrolyte, in terms of the calcium-hydrate suspension, was determined by titration, with phenolphthalein as the indicator. A slight excess of the calcium-hydrate suspension above the amount necessary to neutralize the acid was always added.

TABLE I.—*Effect produced upon the hydrolysis of casein in the presence of starch by varying the time of digestion*

Amino-acid group.	Experiment 1 (10 gm. of casein+300 c. c. of 20 per cent hydrochloric acid; boiled for 12 hours).			Experiment 2 (10 gm. of casein+50 gm. of cornstarch+300 c. c. of 20 per cent hydrochloric acid; boiled for 12 hours).			Experiment 3 (10 gm. of casein+50 gm. of cornstarch+300 c. c. of 20 per cent hydrochloric acid; boiled for 15 hours).			Experiment 4 (10 gm. of casein+50 gm. of cornstarch+300 c. c. of 20 per cent hydrochloric acid; boiled for 24 hours).			Experiment 5 (10 gm. of casein+50 gm. of cornstarch+300 c. c. of 20 per cent hydrochloric acid; boiled for 48 hours).		
	No. 1	No. 2.	Average.	No. 1.	No. 2.	Average.	No. 1.	No. 2.	Average.	No. 1.	No. 2.	Average.	No. 1.	No. 2.	Average.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Ammonia nitrogen	10.20	10.29	10.24	10.31	10.21	10.26	9.46	10.28	9.87	10.09	10.01	10.05	11.29	10.90	11.10
Humin nitrogen	1.32	1.32	1.32	1.47	1.47	1.47	1.10	.75	.92	.95	.95	.95	.74	.80	.77
Arginin nitrogen	7.99	7.90	7.94	6.49	7.06	6.77	5.46	6.21	5.83	6.32	6.23	6.27	6.00	6.00	6.00
Histidin nitrogen	7.94	8.15	8.04	8.18	8.18	8.18	6.93	5.90	6.41	1.19	1.79	1.49	2.71	2.59	2.65
Cystin nitrogen	.14	.17	.15	.19	.16	.17	.15	.19	.17	.07	.06	.07	.07	.05	.06
Lysin nitrogen	9.13	9.50	9.31	8.79	8.82	8.80	8.03	9.47	8.75	10.37	9.85	10.11	11.58	9.88	10.73
Amino nitrogen in filtrate from bases	57.38	57.19	57.28	58.06	56.90	57.48	57.60	57.79	57.69	59.14	58.31	58.72	56.97	56.94	56.95
Nonamino nitrogen in filtrate from bases	8.67	8.26	8.46	8.47	10.31	9.36	11.15	8.93	10.04	8.80	10.17	9.48	8.37	9.69	9.03
Total	102.77	102.78	102.74	101.96	103.11	102.52	99.88	99.52	99.68	97.03	97.47	97.25	97.73	96.85	97.29

The results on humin represent the humin in solution and precipitated by calcium-hydrate suspension. The high results obtained by Grindley, Slater, et al. and Hart and Sure for humin nitrogen were made to include the total nitrogen in the insoluble residue and also the humin in solution.

It has been the experience of the writer that in cases where considerable insoluble residue was included in the total volume of the hydrolyte, great difficulty was met with in obtaining uniform aliquots for the total nitrogen in the solution and also for the aliquot for determination. This difficulty is avoided by filtering out and washing the insoluble residue. Then, too, the question arises, Is it fair to consider the nitrogen remaining in the insoluble residue as humin nitrogen?

The results for arginin show no serious loss in any of the determinations, and the minimum result obtained is only 1.5 per cent below Van Slyke's result for arginin on casein alone.

The histidin results are practically the same for the two 12-hour digestions, on casein alone and on the casein-starch mixture. In the 15-hour casein-starch digestion the result for histidin is 0.61 per cent above that reported in Van Slyke's analysis. In the 24- and 48-hour digestions there is a loss in histidin nitrogen of considerably more than 50 per cent of that found in the 15-hour digestion. Hence, the results for histidin in the two last experiments are very significant, indicating that long periods of digestion of starch and casein bring about a redistribution of the nitrogen in this group. It is to be borne in mind that Hart and Sure[1] obtained similar results on lysin. These writers also report 7.30 per cent as a average for histidin determinations in their experiment.

There is a diminution in the cystin nitrogen of more than 50 per cent in the 24- and 48-hour digestions. Hart and Sure state that their results for cystin were so low that they reported the results obtained by Van Slyke instead.

The results for lysin agree well in the 12- and 15-hour experiments. In the 24- and 48-hour experiments the results for lysin are high. Lysin nitrogen is obtained by deducting the sum of histidin, arginin, and cystin nitrogen from the total nitrogen in the bases; therefore any diminution in the nitrogen content of either histidin, arginin, or cystin will increase the results for lysin nitrogen correspondingly.

There is no marked difference between the results obtained in all the experiments for the amino- and nonamino-nitrogen content in the filtrates from the bases.

In the footings of the different analyses it is to be noted that the 12-hour digestions give footings more than 2.5 per cent over 100. In the 15-hour digestion the footing is good, while in the 24- and 48-hour digestions the footings are 2.75 per cent less than 100, thus indicating that the 12-hour experiments were probably not completely hydrolyzed;

[1] Hart, E. B., and Sure, Barnett. Op. cit.

whereas the 15-hour digestion was sufficient to bring about complete hydrolysis and the 24- and 48-hour experiments were overdigested to the extent that nitrogen was lost.

The insoluble carbonaceous residues which were filtered from the hydrolyzed solutions were dried at 100° C. and the total nitrogen determined in each.

The insoluble residue from experiment 2, or the 12-hour starch-casein digestion, contained 1.30 per cent of nitrogen. That from the 15-hour digestion contained 0.83 per cent of nitrogen. That from the 24-hour digestion contained 0.80 per cent of nitrogen and that from the 48-hour digestion contained 0.855 per cent of nitrogen. The results show that a 15-hour digestion removed as much nitrogen from the insoluble residue as the 24- and 48-hour digestions.

Two determinations of total nitrogen on a sample of the dry starch showed an average nitrogen content of 0.05 per cent. The small amount of nitrogen contained in the starch and the comparatively greater amount found in the insoluble residues indicate that some nitrogen compound was absorbed by the latter.

Seven gm. of the dry-carbon residue were weighed out and transferred to a Claisen flask, 60 c. c. of a 10 per cent calcium-hydrate suspension added, together with 250 c. c. of distilled water. The apparatus was connected up as in an ammonia determination and distilled under reduced pressure at from 40° to 45° C. for 30 minutes. Nine-tenths c. c. of *N/10* hydrochloric acid was neutralized by the ammonia evolved, which shows that the insoluble-carbon residue contained only a trace of ammonia nitrogen. The insoluble-carbon and calcium-hydrate precipitate remaining in the Claisen flask was filtered and washed thoroughly, the filtrate made acid and concentrated under reduced pressure to about 50 c. c. The concentrate was transferred to a Kjeldahl flask and the total nitrogen determined in the usual way. The filtrate contained 0.0032 gm. of nitrogen or 5.3 per cent of the total nitrogen contained in the insoluble residue. The ammonia nitrogen was 2.1 per cent of the total nitrogen in the carbon residue. It is therefore evident that a very small percentage of the total nitrogen contained in the insoluble residue is affected by distilling with calcium-hydrate suspension, which indicates that the nitrogen remaining in the insoluble-carbon residue after digestion and washing is in what may be considered an inert form and should not be included in the humin group.

CONCLUSIONS

From the data contained in this paper the following conclusions may be drawn:

(1) The Van Slyke method for protein analysis, when applied to mixtures of casein and starch in the proportion of 1 to 5, and hydrolyzed

from 12 to 15 hours with 20 per cent hydrochloric acid gives results for the amino-acid groups that are comparable with those obtained by Van Slyke upon casein alone.

(2) A digestion period of more than 15 hours with 20 per cent hydrochloric acid on a casein-starch mixture brings about a redistribution of the nitrogen contained in the histidin and cystin groups.

(3) The insoluble residue obtained from a casein-starch digestion after being thoroughly washed contains nitrogen, which is not seriously affected when distilled with calcium-hydrate suspension, very small amounts being split off as ammonia or remaining in the filtrate. This indicates that the nitrogen is in an inert form and its estimation should not be included in the humin determination.

BEHAVIOR OF SWEET POTATOES IN THE GROUND

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THE PROBLEM

In the course of former investigations[1] on the behavior of sweet potatoes in storage, it was observed that the percentage of starch was always highest and the percentage of sugar lowest in freshly dug potatoes. This observation was more or less incidental, having been made in the course of experiments whose object was the solution of other problems. It was therefore not based upon a systematic study of the roots throughout the latter part of the growing season. Nevertheless the constancy of the condition seemed to justify the conclusion that in the growing sweet potato the reserve materials exist essentially in the form of starch, and that the appearance of sugar in considerable quantities is a phenomenon occuring only in storage or after the destruction of the leaves.

In order to determine whether these quantitative relations between the starch content and the sugar content of the sweet potato remain constant throughout the latter part of the growing season, and to what extent they are changed by the death of the vines, the carbohydrate metabolism in Big Stem sweet potatoes was followed from the time the roots were large enough to furnish the requisite samples until they were seriously damaged by frost.

The record thus obtained of the condition of the potatoes during this period may be useful as an aid in determining the time for harvesting the crop; for it is evident that, if marked changes occur in the roots during the latter part of the season, the time of harvest will depend upon the purpose for which they are destined, whether for storage, stock feed, silage, or, as Keitt has suggested, for the manufacture of starch. In the last case it is evident that the crop should be harvested when the starch content is greatest. As a rule, growers are advised to dig sweet potatoes when they are fully matured or after they have thoroughly ripened. While these phrases imply the idea that the roots reach a more or less definite state of ripeness, the characteristics by which this state may be recognized are not precisely defined. On this matter a record of the

[1] HASSELBRING, Heinrich, and HAWKINS, L. A. PHYSIOLOGICAL CHANGES IN SWEET POTATOES DURING STORAGE. *In* Jour. Agr. Research, v. 3, no. 4, p. 331–342. 1915.

——— ——— CARBOHYDRATE TRANSFORMATIONS IN SWEET POTATOES. *In* Jour. Agr. Research, v. 5, no. 13, p. 543–560. 1915.

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changes in the roots during the latter part of their growth may also throw some light.

PREVIOUS INVESTIGATION

The only systematic examination heretofore published on the behavior of sweet potatoes in the ground is that made in South Carolina by Keitt,[1] who investigated the behavior of four varieties during 1908 and 1909. Since his data are not easily summarized his tables recalculated on the basis of dry matter are given here for purposes of comparison with subsequent data.

TABLE I.—*Percentage composition of sweet potatoes in 1908, according to Keitt*

Date.	Variety.	Water.	Starch.	Glucose.	Sucrose.	Total carbohydrates.
Aug. 28	Nancy Hall	69.23	58.11	1.98	13.58	73.67
Sept. 7	.....do	73.34	55.93	5.93	6.83	68.69
Sept. 18	.....do	69.08	56.95	6.08	6.18	69.21
Sept. 29	.....do	68.70	63.64	7.35	4.57	75.56
Nov. 18	.....do	68.75	57.02	4.90	7.52	69.44
Aug. 28	Polo	70.76	52.94	2.77	14.16	69.87
Sept. 7	.....do	73.85	50.17	6.42	10.75	67.34
Sept. 18	.....do	77.89	51.11	8.68	9.23	69.02
Sept. 29	.....do	70.07	51.69	5.98	1.37	59.04
Nov. 18	.....do	72.53	54.06	6.37	7.35	67.78
Aug. 28	Purple yam	69.79	60.31	4.04	13.31	77.66
Sept. 7	.....do	71.65	54.14	3.67	14.04	71.85
Sept. 18	.....do	70.30	52.86	5.62	9.87	68.35
Sept. 29	.....do	71.30	63.62	7.39	5.64	76.65
Nov. 18	.....do	65.67	56.22	4.19	6.35	66.76
Aug. 28	Fulleton yam	68.40	54.08	3.70	15.98	73.76
Sept. 7	.....do	68.17	52.69	6.57	13.10	72.36
Sept. 18	.....do	67.67	54.38	10.83	6.22	71.43
Sept. 29	.....do	66.72	63.91	7.69	1.86	73.46
Nov. 18	.....do	67.74	58.03	1.61	10.42	70.06

[1] KEITT, T. E. THE FORMATION OF SUGARS AND STARCH IN THE SWEET POTATO. S. C. Agr. Exp. Sta. Bul. 156, 14 p. 1911.

——— SWEET POTATO INVESTIGATION. S. C. Agr. Exp. Sta. Bul. 165, 43 p. 1912.

TABLE II.—*Percentage composition of sweet potatoes in 1909, according to Keitt*

Date.	Variety.	Water.	Starch.	Glucose.	Sucrose.	Total carbohydrates.
Aug. 31	Pumpkin yam	72.45	58.22	13.03	2.50	73.75
Sept. 10	.....do	66.12	57.20	11.98	5.73	74.91
Sept. 21	.....do	74.27	55.03	6.18	8.94	70.15
Oct. 10	.....do	68.34	53.03	3.95	9.95	66.93
Oct. 26	.....do	70.20	59.60	4.50	14.70	78.80
Aug. 31	Purple yam	66.97	63.73	8.27	5.51	77.51
Sept. 10	.....do	64.71	61.06	9.18	3.51	73.75
Sept. 21	.....do	62.42	69.10	3.70	4.76	77.56
Oct. 2	.....do	61.59	69.72	6.77	5.18	81.67
Oct. 10	.....do	60.91	56.46	2.76	7.55	66.77
Oct. 26	.....do	63.83	71.00	4.06	6.50	81.56
Aug. 31	Polo	66.81	70.44	6.87	2.32	79.63
Sept. 10	.....do	68.24	61.52	6.99	8.88	77.39
Sept. 21	.....do	69.44	68.52	6.09	4.16	78.77
Oct. 2	.....do	67.98	64.55	4.18	3.06	71.79
Oct. 10	.....do	61.24	58.38	3.04	6.01	67.43
Oct. 26	.....do	66.08	70.70	3.33	7.61	81.64
Aug. 31	Brazilian	67.45	70.78	7.28	5.74	83.80
Sept. 10	.....do	64.31	62.96	8.21	3.98	75.15
Sept. 21	.....do	65.51	69.99	2.61	7.16	79.76
Oct. 10	.....do	66.22	55.21	3.85	7.70	66.76
Oct. 26	.....do	69.97	65.77	4.86	8.26	78.89

These figures exhibit considerable fluctuation. In 1908 there was as a rule a decrease in the percentage of starch from August 28 to September 18, then a sudden increase from September 18 to September 29, and an equally sudden fall from September 29 to November 18. A frost which killed the vines occurred on November 6. The Polo variety does not conform to the others in its behavior. During the same period all the varieties show a decrease in sucrose up to September 29, and an increase from that time until November 18. The glucose shows a gradual increase, reaching a maximum in the different varieties between September 18 and September 29, after which there is a loss of glucose. During the next year the figures show even greater irregularity. The percentage of starch fell and rose alternately between each pair of successive dates. It is notable that after the frost which killed the vines on October 13, the starch content of all varieties increased considerably. Hence, there is one year a loss of starch after the death of the vines and in the following year an increase. The figures representing the percentages of glucose and sucrose fluctuate irregularly, but in general it may be said that the glucose fell throughout the season, while the sucrose increased. It is to be noted, however, that after the frost both sugars increased. Thus, we have the remarkable phenomenon of an increase in the percentage of of total carbohydrates in the roots after the vines had been killed.

EXPERIMENTAL WORK

In the present investigation Big Stem sweet potatoes were used. They were grown at Bell Station, Maryland, in a sandy field having a gentle slope to the south. Lots of 15 to 20 kgm. were collected each week from September 18 to November 27. The collections were always made in the afternoon. The roots were thoroughly washed and stored in a covered receptacle in the laboratory until the following day, when the samples were prepared for analysis. In each case moisture, starch, cane sugar, and reducing sugar were determined in duplicate in five potatoes of the lot.

At the time of the first digging, September 18, the potatoes were still small, so that it was difficult to get roots large enough to furnish the requisite samples for analysis. After two or three weeks there was an abundance of large roots. The vines remained green and in a healthy state until the week of October 9. Heavy frosts during that week, especially on October 15, killed the leaves but not the stems. By November 7 the stems which remained green after the leaves had been killed were slowly dying, and some of the potatoes showed small round injured spots on the portions near the surface of the ground or projecting above it. On November 21 many of the roots showed considerable injury on the exposed ends, but sound potatoes were still abundant. At the time of the last collection, on November 28, the roots were so extensively damaged that it was difficult to find enough sound ones for analysis. After that date the experiment was discontinued.

The results of the analyses are given in Table III. For purposes of discussion the analytical data have been reduced to the basis of dry matter in the roots. These results are given in the left-hand part of the table. Since it may be desirable to have for reference a record of the actual percentages of the different substances contained in the potatoes, the original analytical data based on the fresh weight of the roots are given in the right-hand part of the table.

TABLE III.—*Percentage composition of Big Stem sweet potatoes in the ground during the latter part of the growing season*

Date.	Sweet potato No.	Moisture.	On the basis of dry matter.				On the basis of fresh material.		
			Starch.	Cane sugar.	Reducing sugar as glucose.	Total carbohydrates.	Starch.	Cane sugar.	Reducing sugar as glucose.
Sept. 18	1	73.38	71.37	7.36	1.20	79.93	19.00	1.96	0.32
	2	75.76	69.72	8.70	1.77	80.19	16.90	2.11	.43
	3	73.70	71.79	7.76	1.14	80.69	18.88	2.04	.30
	4	73.56	72.01	8.17	1.78	81.96	19.04	2.16	.47
	5	73.21	72.08	7.65	1.31	81.04	19.31	2.05	.35
	Average	73.92	71.39	7.93	1.44	80.76	18.63	2.06	.37
Sept. 25	6	73.34	71.53	8.63	1.31	81.47	19.07	2.30	.35
	7	72.35	72.98	7.12	.87	80.97	20.18	1.97	.24
	8	73.99	72.24	6.80	1.19	80.23	18.79	1.77	.31
	9	71.44	74.51	6.72	1.33	82.56	21.28	1.92	.38
	10	75.09	70.69	8.31	1.41	80.41	17.61	2.07	.35
	Average	73.24	72.39	7.52	1.22	81.13	19.39	2.01	.33
Oct. 2	11	74.58	69.04	8.14	1.49	78.67	17.55	2.07	.38
	12	74.76	70.44	9.27	1.62	81.33	17.78	2.34	.41
	13	73.28	71.07	9.96	1.68	82.71	18.99	2.66	.45
	14	71.07	74.59	7.71	1.45	83.75	21.58	2.23	.42
	15	75.64	69.38	8.95	2.38	80.71	16.90	2.18	.58
	Average	73.87	70.90	8.81	1.72	81.43	18.56	2.30	.45
Oct. 9	16	75.69	69.52	8.19	1.81	79.52	16.90	1.99	.44
	17	76.19	68.25	9.83	2.31	80.39	16.25	2.34	.55
	18	75.55	68.83	8.22	2.49	79.54	16.83	2.01	.61
	19	75.30	69.15	8.79	2.23	80.17	17.08	2.17	.55
	20	74.62	70.65	7.88	2.29	80.82	17.93	2.00	.58
	Average	75.47	69.28	8.58	2.23	80.09	17.00	2.10	.55
Oct. 17	21	76.34	69.82	8.37	2.49	80.68	16.52	1.98	.59
	22	76.37	69.53	8.55	3.47	81.55	16.43	2.02	.82
	23	76.57	70.00	7.68	2.99	80.67	16.40	1.80	.70
	24	76.52	68.87	8.05	2.51	79.43	16.17	1.89	.59
	25	75.68	69.00	8.92	2.59	80.51	16.78	2.17	.63
	Average	76.30	69.44	8.31	2.81	80.57	16.46	1.97	.67
Oct. 23	26	77.00	68.70	9.00	2.30	80.00	15.80	2.07	.53
	27	77.43	67.52	9.35	3.06	79.93	15.24	2.11	.69
	28	76.18	70.07	8.61	2.06	80.74	16.69	2.05	.49
	29	78.87	67.87	8.00	3.69	79.56	14.34	1.69	.78
	30	77.66	68.40	9.36	2.91	80.67	15.28	2.09	.65
	Average	77.43	68.51	8.86	2.80	80.18	15.47	2.00	.63

TABLE III.—*Percentage composition of Big Stem sweet potatoes in the ground during the latter part of the growing season*—Continued

Date.	Sweet potato No.	Moisture.	On the basis of dry matter.				On the basis of fresh material.		
			Starch.	Cane sugar.	Reducing sugar as glucose.	Total carbohydrates.	Starch.	Cane sugar.	Reducing sugar as glucose.
Oct. 30	31	77.00	65.52	10.65	2.87	79.04	15.07	2.45	0.66
	32	77.91	65.05	11.77	2.81	79.63	14.37	2.60	.62
	33	77.66	65.22	10.56	2.91	78.69	14.57	2.36	.65
	34	78.79	59.74	14.47	4.24	78.45	12.67	3.07	.90
	35	77.52	67.70	8.76	2.98	79.44	15.22	1.97	.67
	Average	77.78	64.65	11.24	3.16	79.05	14.38	2.49	.70
Nov. 6	36	76.17	62.82	14.65	3.40	80.87	14.97	3.49	.81
	37	76.11	64.92	14.15	2.68	81.75	15.51	3.38	.64
	38	76.59	61.90	14.44	2.95	79.29	14.49	3.38	.69
	39	74.71	65.01	11.82	1.86	78.69	16.44	2.99	.47
	40	76.37	63.27	13.67	2.58	79.52	14.95	3.23	.61
	Average	75.99	63.58	13.75	2.69	80.02	15.27	3.29	.64
Nov. 13	41	78.02	59.55	15.24	2.59	77.38	13.09	3.35	.57
	42	78.23	54.89	18.56	2.99	76.44	11.95	4.04	.65
	43	77.14	57.13	18.20	2.45	77.78	13.06	4.16	.56
	44	78.83	51.54	20.08	3.40	75.02	10.91	4.25	.72
	45	78.32	53.23	19.93	5.03	78.19	11.54	4.32	1.09
	Average	78.14	55.27	18.40	3.29	76.96	12.11	4.02	.72
Nov. 20	46	76.99	52.41	20.99	3.30	76.70	12.06	4.83	.76
	47	79.22	48.32	25.31	3.75	77.38	10.04	5.26	.78
	48	76.02	55.46	19.72	2.79	77.97	13.30	4.73	.67
	49	79.45	46.76	25.16	3.41	75.33	9.61	5.17	.70
	50	77.20	50.70	24.39	2.76	77.85	11.56	5.56	.63
	Average	77.78	50.73	23.11	3.20	77.03	11.31	5.11	.71
Nov. 27	51	79.93	43.70	28.10	5.08	76.88	8.77	5.64	1.02
	52	78.64	47.85	26.59	3.60	78.04	10.22	5.68	.77
	53	79.71	44.36	26.61	4.04	75.01	9.00	5.40	.82
	54	78.37	47.06	25.52	3.61	76.19	10.18	5.52	.78
	55	77.98	48.05	23.48	2.91	74.44	10.58	5.17	.64
	Average	78.93	46.20	26.06	3.85	76.11	9.75	5.48	.81

An examination of Table III shows that the sweet potatoes exhibit some individual fluctuation in composition, but these variations are not sufficiently great to obscure the seasonal trend. They show, however, that small deviations in the general contour of the seasonal changes are to be expected where so small a number of individuals is examined. The significant changes lie clearly outside the limits of the individual fluctuations. The seasonal changes in the percentage of the various constituents of the sweet potato as shown by the table are briefly described below.

Moisture.—The moisture content of the roots remained almost uniform during the period covered by the first three collections, from September 18 to October 2. After that time there was a gradual increase in the percentage of moisture until the end of the season. A small fluctuation appears in the lot collected on November 6. The changes in water content of the roots are therefore fairly regular and uniform. No such marked fluctuations as those recorded in the tables of Keitt are evident from these data. The beginning of the accumulation of moisture in the roots is practically coincident with the destruction of the leaves.

Starch.—The percentage of starch in the dry matter of the roots shows a slight decrease from September 18 until October 23, varying during that period between 71.39 per cent and 68.51 per cent. On October 30, the date of the next collection, the starch content had fallen to 64.65 per cent. From that date the starch content continued to fall until the end of the season, when the minimum of 46.20 per cent was reached. The rapid disappearance of starch follows, somewhat delayed, upon the death of the leaves.

Cane sugar.—The cane-sugar content remains practically constant between 7.52 and 8.86 per cent until the time when the percentage of starch begins to fall rapidly. With the decrease in starch the cane sugar begins to increase correspondingly until it finally represents 26.06 per cent of the dry matter of the roots. It should be noted, however, that in general the changes in cane sugar are inaugurated somewhat later than those of the starch.

Reducing sugar.—The reducing sugar content remains constant at first and then shows a gradual rise until the final percentage is somewhat more than double that at the beginning. The increase in reducing sugar antecedes somewhat the rise in cane sugar. It is noteworthy also that under these conditions reducing sugar apparently does not accumulate to the same extent to which it accumulates in sweet potatoes in storage.

Total carbohydrates.—The total carbohydrate content undergoes very little change until toward the end of the season, when the roots begin to show marked injury by frost. At that time an evident loss of carbohydrates becomes apparent. The constancy of the total carbohydrate content of the sweet potato is in marked contrast to the fluctuations observed by Keitt.

The foregoing facts have been embodied in the curves in figure 1. The curves are based on the averages of the analyses for each week.

CONCLUSIONS

It may be concluded from this investigation that the changes occurring in sweet potatoes in the ground during the later part of the growing season proceed in a regular and orderly manner. During the later part of the period of growth the composition of the roots remains remarkably uniform, and presents no striking or irregular fluctuations. During this

period the root is characterized by a high starch content, and a low sugar content. The changes which occur later are associated with the death of the vines. Prominent among these changes is the accumulation of water in the roots as a result of the cessation of transpiration in conse-

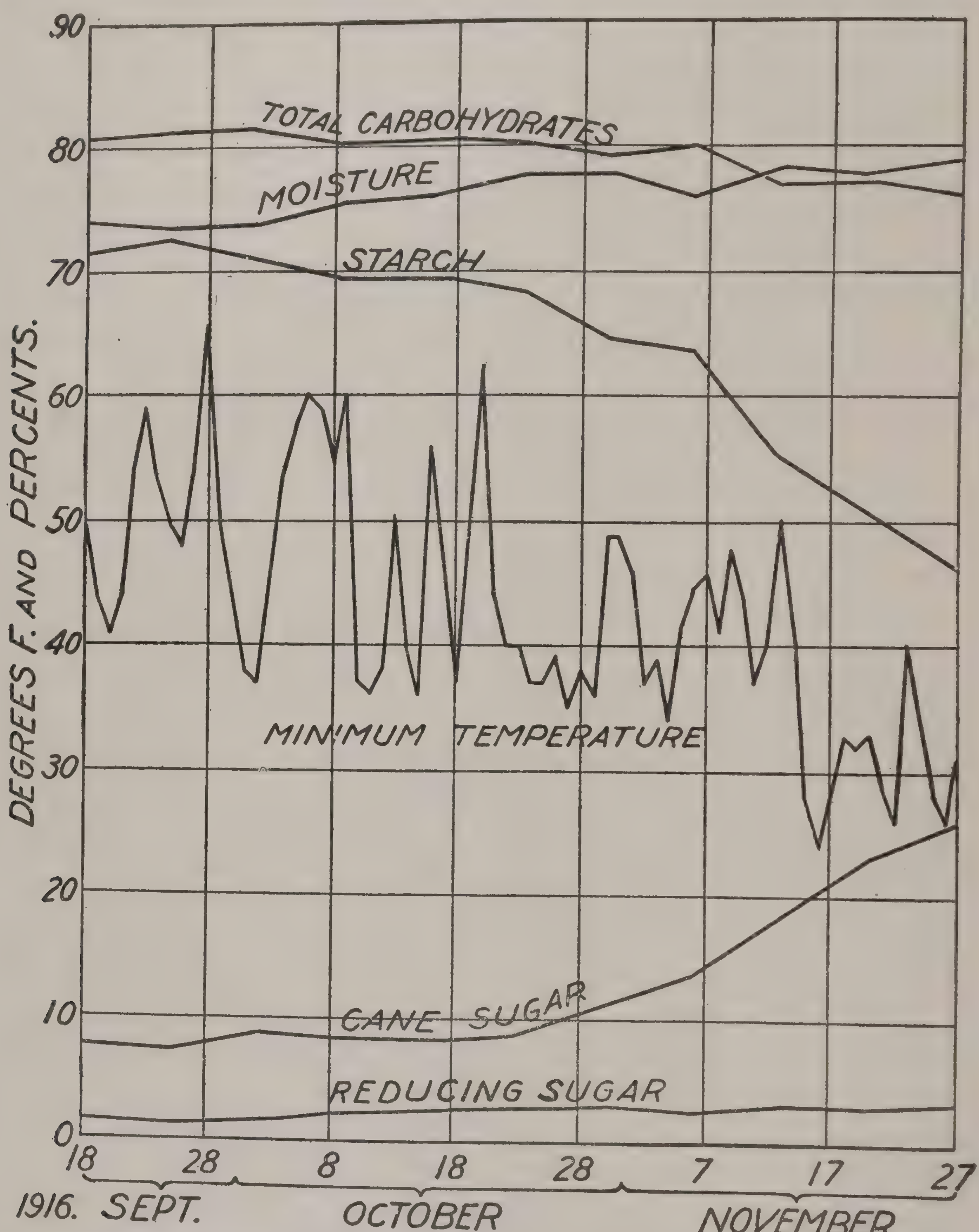

FIG. 1.—Graphs showing changes in composition of Big Stem sweet potatoes during the latter part of the season, from September 18 to November 27, and the minimum temperatures at the United States Weather Bureau Observatory at Washington, D. C., some 20 miles distant, during that period. The ordinates indicate percentages in the one case and degrees Fahrenheit in the other.

quence of the destruction of the leaves. With the termination of the flow of materials from the vines the carbohydrate transformations characteristic of sweet potatoes in storage are inaugurated. These changes consist in the transformation of starch into sugars. In point

of time the decrease in starch and the increase in reducing sugar precede somewhat the increase in cane sugar. It appears, therefore, that reducing sugar is formed first as an intermediate step in the change from starch to cane sugar. The loss caused by respiration, which is considerable during the curing process and in storage, is apparently slight in sweet potatoes in the ground. Appreciable destruction of carbohydrates appears not to occur under these conditions until late in the season when the roots have been injured by frosts.

The changes here described have a practical bearing on the question of maturation of sweet potatoes and on the choice of the time of harvest. Since the carbohydrate relations of the roots in the ground remain practically unchanged while the vines are uninjured, the roots can not be said to undergo a definite process of ripening, in the sense of a progressive transformation of one reserve substance into another, such as the change of insoluble pectin into soluble pectin derivatives in the peach, or the transformation of starch into cane sugar and invert sugar in the apple. Under ordinary conditions the potatoes continue to grow until frost without reaching any definite state of maturity recognizable by progressive changes in the reserve materials which they contain. The changes in storage, which may perhaps be regarded as a process of maturation, do not come in for consideration here, since statements relating to the degree of maturity of sweet potatoes always refer to the growing roots. It is evident from these considerations that the choice of time of harvest is not a matter of maturity of the roots, but is governed by other factors. The potatoes may safely be kept in the ground until the leaves have been injured by frost.

Of the changes which occur after the destruction of the leaves, the accumulation of water in the roots deserves foremost consideration. It can scarcely be doubted that this increased water content is detrimental to the successful storage of the roots, and causes them to be more subject to decay than roots of normal water content. One of the objects of the rather expensive operation of curing is to eliminate a part of the water contained in the roots. As a rule observers agree that cured sweet potatoes keep better than uncured ones. Only occasionally a statement to the contrary is found. It may therefore safely be assumed that the increase in the relative proportion of water in the roots will be detrimental to storage. On this account it is of utmost importance that the harvesting of sweet potatoes be not long delayed after the leaves have been killed by frost. The other changes occurring in sweet potatoes in the ground are essentially the same as the changes occurring in storage. These changes are therefore in no way detrimental to the crop, since no appreciable loss of carbohydrates occurs until the roots have been so severely injured that they have lost their market value.

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STUDIES IN SOIL REACTION AS INDICATED BY THE HYDROGEN ELECTRODE

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INTRODUCTION

There has been developed in the past few years a rather voluminous literature dealing with the subject of soil reaction. One has only to scan this literature in order to find wide variations of opinion between investigators as to the cause and nature of soil acidity.

Until recently the various qualitative and quantitative methods in vogue for indicating soil acidity or lime requirement have not been sufficiently delicate to draw definite conclusions as to the "true reaction" of soils. The lack of uniformity and accuracy of methods has undoubtedly caused such confusion on this subject. By adopting modern methods for measuring soil reaction many of the contentions should be obliterated.

Though the hydrogen electrode has been used for some time in indicating changes in reaction, Gillespie (*7*) [1] was the first to use it on an extended scale as an indicator of reaction in soils. Sharp and Hoagland (*12*) have since measured the H-ion concentration of a number of oils in suspension, and have extended this method to studying other soil phenomena.

The significance of the terms "true acidity," "true alkalinity," and "true neutrality" need not be defined here, except in so far as an explanation of the method adopted in reporting results obtained. Pure water dissociates into H and OH ions in equal concentration. The product of the concentration of these ions in a solution is a constant, approximately $1 \times 10-14$. When the H ions are present in a concentration greater than $1 \times 10-7$, the solution is acid; the presence of OH ions in greater concentrations than $1 \times 10-7$ results in an alkaline solution. For a more detailed discussion of this subject, the reader is referred to texts on electrochemistry.

The investigations herein reported were begun about the time of the appearance of Sharp and Hoagland's paper, to ascertain if appreciable differences occurred in the H-ion concentration of soils of humid regions, especially from those of the Southern States. It would appear that with the excessive rainfall of this region an accumulation of soluble acids in the soil would be almost impossible.

[1] Reference is made by number (italic) to "Literature cited," p. 30-31.

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The method has been used for indicating the reaction of the soil film water. The effects of several fertilizer materials have been studied on the H-ion concentration of various field soils, receiving applications of such fertilizers for a number of years. Lastly, the effect of ammonium sulphate and monocalcium phosphate on the reaction of soil film water have been investigated.

METHODS OF INVESTIGATION

The apparatus for measuring the H-ion concentration of soil suspensions and extracts was essentially that described by Hildebrand (*8*), and modified by Sharp and Hoagland. Palladium was substituted for platinum as the electrode, which was treated in a manner similar to that described by Findlay (*6*) for coating electrodes. The supply of hydrogen was obtained by electrolyzing water, with potassium hydroxid as the electrolyte. All the precautions of rigidity of connections, insulation of apparatus, time (which often varied) for establishment of equilibrium between soil and solution, coating electrodes, and prevention of loss of CO_2 were strictly observed.

It might be said in passing that no difficulties were encountered due to the reduction of nitrates to ammonia, as has been suggested.

In the preparation of soil suspensions, unless otherwise stated, the arbitrary ratio of 10 gm. of air-dried soil to 100 c. c. of as pure distilled water as obtainable was used. In the case of field soils their content was determined as soon after sampling as practicable, and the same ratio of soil to water maintained. All samples except those taken directly from the field were screened through a 2-mm. sieve.

In all cases the readings became constant in a few minutes. Duplicate readings on the same sample of soil could easily be read to 0.02 volt. However, to be certain that equilibrium had been established the electrode was allowed to remain in contact with solution for 30 minutes. It was almost impossible to get such closely agreeing results as 0.02 volt with different samples of the same soil. This can be accounted for in the lack of uniformity of mixing.

The results are reported in the usual manner for such measurements, units of gram-molecules of H ion per liter. The tables of Schmidt (*11*) were used in securing the H-ion concentration from the voltmeter readings.

RESULTS OBTAINED

In Table I will be found the results of measurements of the H-ion concentrations of 68 samples of untreated soils, including subsoils, which represent a wide range in types of five series. Included in this table are results derived from five samples of treated soil. The samples are taken as a fair representation of the soils common to the area of the southeastern portion of the United States, which extends from and including the Appalachian Mountains to the Atlantic Ocean.

TABLE I.—*Hydrogen-ion concentration of soil suspensions*

Sample No.	Soil type.[a]	Depth.	Volt-meter readings.	H-ion concentration.
		Inches.		*Gram molecules per liter.*
1565	Norfolk sand (sand-hill phase)	0–4	0.700	0.4×10^{-6}
1566	.....do	4–21	.684	$.9\times10^{-6}$
1067	.....do	0–4	.712	$.3\times10^{-6}$
1068	.....do	4–18	.706	$.3\times10^{-6}$
1501	Norfolk coarse sandy loam	0–6	.684	$.9\times10^{-6}$
1502	.....do	6–28	.684	$.9\times10^{-6}$
1287	.....do	0.6	.696	$.5\times10^{-6}$
1288	.....do	6–26	.681	1.0×10^{-6}
1397	Norfolk sandy loam	0–7	.674	$.1\times10^{-5}$
1398	.....do	7–28	.668	$.1\times10^{-5}$
1483	.....do	0–7	.662	$.2\times10^{-5}$
1484	.....do	7–28	.674	$.1\times10^{-5}$
1344	Norfolk fine sandy loam	0–6	.660	$.2\times10^{-5}$
1345	.....do	6–36	.671	$.1\times10^{-5}$
991	.....do	0–6	.645	$.4\times10^{-5}$
992	.....do	6–36	.640	$.5\times10^{-5}$
1551	Norfolk very fine sandy loam	0–6	.657	$.2\times10^{-5}$
1552	.....do	6–24	.660	$.2\times10^{-5}$
1029	.....do	0–8	.614	$.1\times10^{-4}$
1030	.....do	8–30	.583	$.5\times10^{-4}$
1519	Norfolk silt loam	0–6	.574	$.7\times10^{-4}$
1520	.....do	6–28	.568	$.9\times10^{-4}$
1499	.....do	0–6	.560	$.1\times10^{-3}$
1500	.....do	6–32	.555	$.1\times10^{-3}$
1352	Cecil coarse sandy loam	0–7	.712	$.3\times10^{-6}$
1353	.....do	7–20	.699	$.5\times10^{-6}$
1287	.....do	0–6	.706	$.3\times10^{-6}$
1288	.....do	6–30	.700	$.4\times10^{-6}$
1125	Cecil fine sandy loam	0–8	.702	$.4\times10^{-6}$
1126	.....do	8–24	.681	1.0×10^{-6}
1261	.....do	0–8	.699	$.4\times10^{-6}$
1262	.....do	8–26	.673	$.1\times10^{-5}$
1336	Cecil clay loam	0–6	.652	$.3\times10^{-5}$
1337	.....do	6–30	.666	$.1\times10^{-5}$
1205	.....do	0–6	.659	$.2\times10^{-5}$
1333	.....do	6–36	.643	$.5\times10^{-5}$
1322	Cecil clay	0–7	.641	$.5\times10^{-5}$
1323	.....do	7–20	.582	$.5\times10^{-4}$
1435	.....do	0–6	.646	$.4\times10^{-5}$
1436	.....do	6–36	.607	$.1\times10^{-4}$
1121	Iredell fine sandy loam	0–6	.732	$.1\times10^{-6}$
1122	.....do	6–36	.738	$.1\times10^{-6}$
1344	.....do	0–8	.711	$.3\times10^{-6}$
1345	.....do	8–24	.704	$.4\times10^{-6}$
1328	Iredell loam	0–8	.692	$.6\times10^{-6}$
1329	.....do	8–24	.714	$.2\times10^{-6}$
1257	.....do	0–6	.684	$.9\times10^{-6}$
1258	.....do	6–26	.679	$.1\times10^{-5}$
395	Porter's sand	0–6	.673	$.1\times10^{-5}$
396	.....do	6–20	.668	$.1\times10^{-5}$
397	.....do	0–6	.640	$.5\times10^{-5}$
398	.....do	6–20	.638	$.5\times10^{-5}$
639	Porter's sandy loam	0–7	.648	$.3\times10^{-5}$
640	.....do	7–28	.641	$.5\times10^{-5}$
6652	.....do	0–7	.656	$.2\times10^{-5}$
653	.....do	7–30	.649	$.3\times10^{-5}$
1201	Porter's loam	0–8	.644	$.4\times10^{-5}$

[a] The writer is indebted to Dr. W. H. McIntire, of the Tennessee Agricultural Experiment Station, for the samples of the treated soils.

TABLE I.—*Hydrogen-ion concentration of soil suspensions*—Continued

Sample No.	Soil type.	Depth.	Volt-meter readings.	H-ion concentration.
		Inches.		*Gram molecules per liter.*
1202	Porter's loam	8–30	.639	$.5\times10^{-5}$
1372	.....do	0–7	.647	$.3\times10^{-5}$
1373	.....do	7–24	.639	$.5\times10^{-5}$
674	Porter's clay	0–7	.577	$.6\times10^{-4}$
675	.....do	7–24	.566	$.1\times10^{-3}$
684	.....do	0–6	.594	$.3\times10^{-4}$
685	.....do	6–20	.587	$.4\times10^{-4}$
1007	Muck	0–18	.483	$.2\times10^{-2}$
1212	.....do	0–24	.462	$.6\times10^{-2}$
1077	.....do	0–36	.536	$.3\times10^{-3}$
2526	.....do	0–36	.427	$.2\times10^{-1}$
	Chickamauga limestone soil [a]		.864	$.7\times10^{-9}$
	Chickamauga limestone soil [b]		.824	$.3\times10^{-8}$
	Cumberland loam [a]		.893	$.2\times10^{-9}$
	Cumberland loam [b]		.856	$.9\times10^{-9}$
	Tillico sandy loam [a]		.866	$.6\times10^{-9}$

[a] Treated in 1912 with 16,000 pounds of calcium carbonate per acre in excess of Vietch indication.
[b] Treated in 1912 with $MgCO_3$-$CaCO_3$ at rate of 16,000 pounds per acre in excess of Vietch indication.

The results shown in Table I indicate wide variations in the H-ion concentrations of the untreated soils under experiment. This varies from very nearly neutral in some of those of the Iredell series to rather excessive acidity in the Norfolk silt loam and mucks. This is in accord with what would be expected. The Iredell soils are of residual origin formed from basic eruptions, mainly diorite. The amount of basic elements supplied this soil is greatly in excess of those commonly found in the area included in this study. The Norfolk silt loam being a transported soil, was formed under conditions through which the basic elements have been removed. It also contains rather high amounts of partially decomposed organic matter, and should show a high concentration of H over OH ions. Field and pot tests with various crops have shown indications of excessive acidity on some of the muck soils. Indeed on some of the muck fields from which the samples were taken little or none of the common agricultural crops will thrive until the land has been limed.

No definite relationship appears to exist between the H-ion concentration and types of different texture. It can be noticed that there is a tendency for the H ion to increase as the number of fine particles increase in a series. However, there are exceptions. The clay loams and loams of the Iredell and Porter's series show greater H-ion concentration than do those of coarser texture. It may be observed that in many cases the subsoil shows a greater degree of acidity than the corresponding surface soil. The general practice in farming these soils has been the removal from the land of all crops. Probably there is a tendency for plant roots to remove more bases from lower depths than from the surface.

The samples of soil which have been treated with excessive amounts of calcium and magnesium carbonate show a greater concentration of OH than H ions in solution. Dr. McIntire informed the writer that the carbonates have long since disappeared in these soils. Obviously the new compounds of calcium and magnesium, whatever they may be, give a strong basic reaction.

These results bear out very forcibly the contentions of Gillespie (7) and Sharp and Hoagland (*12*) that there is a preponderance of H over OH ions in the liquid phase of many soil suspensions. Whether the "true acidity" as indicated from the results of Table I is developed from organic acids or acid silicates can not be stated. In some of the sandy soils the organic matter is quite low, yet indications lead to the conclusion that there is a greater concentration of H ions than found in neutral solutions. With the muck samples the organic-matter content is very high, being 90 per cent or more, and most marked acidity is shown.

H-ION CONCENTRATION OF SOIL FILM OR CAPILLARY WATER

The question has often been raised, "Is the water held as a liquid film around the soil particles of different reaction from that in the free state?" The difficulty encountered in securing any workable amount of the film water has militated against any direct study of this question. Recently, Morgan (*9*) has devised a workable modification of the "oil displacement" method for obtaining the soil solution in an unaltered form. A study of this water should throw much light on some of the obscure problems of soils. One serious difficulty yet remains with the method, which is its inability to get back all of water held as thin films around the soil grains. However, a study of the reaction of that portion of the film water obtainable should give indications of value as to the conditions of the soil solution.

The Morgan apparatus, with a few unimportant modifications, has been used in obtaining that portion of the capillary water displaced by paraffin oil under high pressure. The oil used in this work was the purest obtainable. As far as could be noticed, it gave a neutral reaction with the hydrogen-electrode apparatus and by titrating against standard alkali with methyl orange, methyl red, and phenolpthalein as indicators.

Some of the soil samples used were taken directly from the filed; others had been stored in the laboratory for a number of years. The portion which passed a 2-mm. sieve was thoroughly mixed, and the water content determined. Sufficient distilled water was added to bring the moisture content up to about what is considered the optimum for that soil. Water lost through evaporation was replaced at frequent intervals, after which the soils were thoroughly packed in the cylinder and treated with oil under high pressure.

The results of this investigation are shown in Table II

TABLE II.—*Reaction of soil-film water*

Soil type.	Film water.					Free water.			Voltmeter readings.	H-ion concentration.
	Amount of soil.	Moisture content (per liter dry basis).	Moisture recovered.	Time of contact.	Volume of film water.	Amount of soil.	Amount of water.	Time of contact.		
	Lbs.	*P. ct.*	*P. ct.*	*Days.*	*C. .c*	*Gm.*	*C. c.*	*Days.*		*Gram-molecules per liter.*
Cecil clay loam	10	25	7.2	4	50				0.572	$0.8+10^{-4}$
Do	10	25	6.9	4	50				.588	$.4+10^{-4}$
Do						5	50	4	.679	$.1+10^{-5}$
Porter's loam	10	25	6.4	4	50				.593	$.3+10^{-4}$
Do	10	25	4.9	4	50				.604	$.2+10^{-4}$
Do						5	50	4	.684	$.9+10^{-6}$
Norfolk fine sandy loam	10	20	15.3	3	50				.614	$.1+10^{-4}$
Do	10	20	13.3	3	50				.624	$.1+10^{-4}$
Do						5	50	3	.661	$.2+10^{-5}$
Norfolk sand	10	15	18.4	2	50				.666	$.1+10^{-5}$
Do	10	15	20.6	2	50				.654	$.3+10^{-5}$
Do						5	50	2	.694	$.6+10^{-6}$
Tellico sandy loam [a]	5	20	12.8	3	25				.929	$.5+10^{-10}$
Do						2.5	25	3	.877	$.4+10^{-9}$
Chickamauga limestone soil [a]	5	25	7.7	3	25				.976	$.8+10^{-11}$
Do						2.5	25	3	.896	$.1+10^{-9}$

[a] Sufficient soil not available for duplicate extractions.

The data presented in Table II show rather conclusively that the soil-film water has the same reaction as the free water. The difference is only in degree of intensity. Those soils which showed an acid reaction in suspensions gave a greater concentration of H ions in the film water. Conversely, those which indicated a greater OH-ion concentration than H in suspension gave a greater intensity of OH ions in the solution. These results are not in keeping with those of Sharp and Hoagland (*12*), who found no appreciable change in H or OH ions in varying the proportions of soil to water in making suspensions.

With the freezing-point method for measuring the concentration of the soil solution Bouyoucos and McCool (*3*) show that as the amount of water decreases in an arithmetic progression the lowering of the freezing point (increase in concentration) increases in a geometric progression. This apparently is due to the rendering inactive of a portion of the water in the soils, hence this water does not take part in dissolving the solutes of the soil. That portion left free or uncombined becomes highly charged with soluble salts and gives phenomenal increases in the freezing-point lowering. The same line of reasoning may be applied to the increase in intensity of reaction of film water when compared to that of soil suspensions.

With the heavier types of soil only a small percentage of the added water was recovered. It would be of extreme interest to note the intensity of reaction of the thinnest moisture film which could remain in contact with the soil grains. In other words, all of the liquid added to the soil should be recovered and studied.

EFFECT OF FERTILIZER MATERIALS ON SOIL REACTION

It has frequently been stated that certain fertilizer materials, more especially ammonium sulphate and acid phosphate, increase the acidity of soils. To obtain additional evidence, the H-ion concentration of soil suspensions have been measured in samples taken from the fertilizer plots of the North Carolina Experiment Station. Some of the soils have received annual applications of these fertilizers for as many as 15 years. All fields are located on well-defined soil types for each area of the State. Three distinct types have been studied.

The samples of both soil and subsoil were collected as carefully as possible. Borings were made at several points on the plots for both surface and subsoil samples. These were taken to the laboratory as quickly as possible and thoroughly mixed, after which water was determined in each composite sample. The ratio of water to soil was kept the same as with the air-dried soils.

Table III gives a compilation of the total amount of each fertilizer material and lime which has been applied to the plots at the different branch stations.

Table III.—*Total quantity (in pounds per acre) of fertilizer materials applied to experimental plots*

Branch station.	Ammonium sulphate (20 per cent nitrogen).	Sodium nitrate (14 per cent nitrogen).	Dried blood (14 per cent nitrogen).	Acid phosphate (16 per cent phosphorus pentoxid).	Potassium sulphate, (40 per cent potassium oxid).	Lime (90 per cent calcium carbonate).
Buncombe	....	....	4,826	1,846	1,100	6,000
Iredell	577	675	1,288	3,332	540	4,000
Central	577	675	....	3,237	307	4,000
Edgecombe	577	675	1,638	3,372	457	4,000

The data secured from the measurements of the H-ion concentration of plots fertilized with ammonium sulphate and sodium nitrate are given in Table IV.

Marked increases in acidity can be noticed in samples taken from plots fertilized with ammonium sulphate. In every case pronounced increase of H ions is evident, and extends to the subsoil in all of the fields studied. On the Norfolk fine sandy loam more acidity is noted in the subsoil than surface.

There does not appear to be any relationship between texture and development of acidity by ammonium sulphate. This is in agreement with the work of Allison and Cook (*1*).

The results secured from the sodium-nitrate plots are in harmony with the accepted theory regarding its effect on soil reaction. A reduction of acidity is apparent.

TABLE IV.—*Effect of ammonium sulphate and nitrate of soda on the H-ion concentration of field-soil suspensions*

Branch station.	Year of beginning experiment.	Soil type.	Depth.	Treatment.	Voltmeter readings.	H-ion concentration.
			Inches.			*Gram-molecules per liter.*
Iredell	1907	Cecil clay loam	0–6	Ammonium sulphate	0.542	0.2×10⁻³
Do	1907	do	6–24	do	.568	.9×10⁻⁴
Do	1907	do	0–6	Sodium nitrate	.694	.6×10⁻⁶
Do	1907	do	6–24	do	.678	.1×10⁻⁶
Do	1907	do	0–6	Untreated	.656	.2×10⁻⁵
Do	1907	do	6–24	do	.649	.3×10⁻⁶
Central	1907	Durham sandy loam	0–8	Ammonium sulphate	.548	.2×10⁻³
Do	1907	do	8–24	do	.582	.5×10⁻⁴
Do	1907	do	0–8	Sodium nitrate	.671	.1×10⁻⁵
Do	1907	do	8–24	do	.656	.2×10⁻⁵
Do	1907	do	0–8	Untreated	.613	.1×10⁻⁴
Do	1907	do	8–24	do	.603	.2×10⁻⁴
Edgecombe	1907	Norfolk fine sandy loam	0–6	Ammonium sulphate	.556	.1×10⁻³
Do	1907	do	6–24	do	.548	.2×10⁻³
Do	1907	do	0–6	Sodium nitrate	.666	.1×10⁻⁵
Do	1907	do	6–24	do	.648	.3×10⁻⁵
Do	1907	do	0–6	Untreated	.646	.4×10⁻⁵
Do	1907	do	6–24	do	.628	.8×10⁻⁵

EFFECT OF POTASSIUM SULPHATE ON SOIL REACTION

Skinner and Beattie (*13*) and others have observed that potassium sulphate increased the lime requirement of soils. Measurements have been made of the H-ion concentration of soil suspensions from plots to which have been added different amounts of this salt. These data are given in Table V.

TABLE V.—*Effect of potassium sulphate on the H-ion concentration of field-soil suspensions*

Branch station.	Year of beginning experiment.	Soil type.	Depth.	Treatment.	Voltmeter readings.	H-ion concentration.
			Inches.			*Gram-molecules per liter.*
Buncombe	1911	Porter's loam	0–8	Dried blood	0.656	0.2×10⁻⁵
Do	1911	do	8–24	do	.654	.3×10⁻⁵
Do	1911	do	0–8	Dried blood, potassium sulphate.	.623	.1×10⁻⁴
Do	1911	do	8–24	do	.623	.1×10⁻⁴
Do	1911	do	0–8	Potassium sulphate	.609	.1×10⁻⁴
Do	1911	do	8–24	do	.596	.3×10⁻⁴
Do	1911	do	0–8	Untreated	.648	.3×10⁻⁵
Do	1911	do	8–24	do	.651	.3×10⁻⁵
Iredell	1903	Cecil clay loam	0–8	Dried blood	.682	.1×10⁻⁶
Do	1903	do	8–36	do	.671	.1×10⁻⁵
Do	1903	do	0–8	Dried blood, potassium sulphate.	.630	.8×10⁻⁶
Do	1903	do	8–36	do	.618	.1×10⁻⁴
Do	1903	do	0–8	Potassium sulphate	.621	.1×10⁻⁴
Do	1903	do	8–36	do	.608	.1×10⁻⁴
Do	1903	do	0–8	Untreated	.676	.1×10⁻⁵
Do	1903	do	0–36	do	.672	.1×10⁻⁶
Edgecombe	1903	Norfolk fine sandy loam	0–8	Dried blood	.632	.7×10⁻⁵
Do	1903	do	8–24	do	.620	.1×10⁻⁴
Do	1903	do	0–8	Dried blood, potassium sulphate.	.602	.2×10⁻⁴
Do	1903	do	8–24	do	.590	.3×10⁻⁴
Do	1903	do	0–8	Potassium sulphate	.596	.3×10⁻⁴
Do	1903	do	8–24	do	.600	.2×10⁻⁴
Do	1903	do	0–8	Untreated	.634	.6×10⁻⁶
Do	1903	do	8–24	do	.628	.8×10⁻⁶

Slight increase in the H-ion concentration was obtained from the plots to which potassium sulphate had been applied. However, nothing like as marked an effect in producing "true acidity" is found on these plots as those to which have been added ammonium sulphate. Why this should be is not clear, unless it has been caused by nitric acid developed by the soil organisms.

EFFECT OF ACID PHOSPHATE AND LIME ON SOIL REACTION

Table VI gives the results from the plots which have received annual applications of acid phosphate and lime.

TABLE VI.—*Effect of acid phosphate and lime on the H-ion concentration of field-soil suspensions*

Branch station.	Year of beginning experiment.	Soil type.	Depth.	Treatment.	Voltmeter readings.	H-ion concentration.
			Inches.			*Gram-molecules per liter.*
Buncombe	1911	Porter's loam	0–8	Dried blood	0.656	0.2×10^{-5}
Do	1911	do	8–30	do	.654	$.3\times10^{-5}$
Do	1911	do	0–8	Acid phosphate	.662	$.2\times10^{-5}$
Do	1911	do	8–30	do	.655	$.2\times10^{-5}$
Do	1911	do	0–8	Dried blood, acid phosphate.	.652	$.3\times10^{-5}$
Do	1911	do	8–30	do	.660	$.2\times10^{-5}$
Do	1911	do	0–8	Dried blood, acid phosphate, potassium sulphate.	.612	$.1\times10^{-4}$
Do	1911	do	8–30	do	.610	$.1\times10^{-5}$
Do	1911	do	0–8	Dried blood, acid phosphate, potassium sulphate, lime.	.702	$.4\times10^{-6}$
Do	1911	do	8–30	do	.716	$.2\times10^{-6}$
Do	1911	do	0–8	Lime	.728	$.1\times10^{-6}$
Do	1911	do	8–30	do	.736	$.1\times10^{-6}$
Do	1911	do	0–8	Untreated	.648	$.3\times10^{-5}$
Do	1911	do	8–30	do	.651	$.3\times10^{-5}$
Iredell	1903	Cecil clay loam	0–6	Dried blood	.682	1.0×10^{-6}
Do	1903	do	6–30	do	.671	$.1\times10^{-5}$
Do	1903	do	0–6	Acid phosphate	.674	$.1\times10^{-5}$
Do	1903	do	6–30	do	.679	$.1\times10^{-5}$
Do	1903	do	0–6	Dried blood, acid phosphate.	.684	$.9\times10^{-6}$
Do	1903	do	6–30	do	.666	$.1\times10^{-5}$
Do	1903	do	0–6	Dried blood, acid phosphate, potassium sulphate.	.613	$.1\times10^{-4}$
Do	1903	do	6–30	do	.609	$.1\times10^{-4}$
Do	1903	do	0–6	Dried blood, acid phosphate, potassium sulphate, lime.	.712	$.3\times10^{-6}$
Do	1903	do	6–30	do	.732	$.1\times10^{-6}$
Do	1903	do	0–6	Lime	.746	$.7\times10^{-7}$
Do	1903	do	6–30	do	.754	$.5\times10^{-7}$
Do	1903	do	0–6	Untreated	.666	$.1\times10^{-5}$
Do	1903	do	6–30	do	.651	$.3\times10^{-5}$
Edgecombe	1903	Norfolk fine sandy loam.	0–6	Dried blood	.632	$.7\times10^{-5}$
Do	1903	do	6–24	do	.620	$.1\times10^{-4}$
Do	1903	do	0–6	Acid phosphate	.624	$.1\times10^{-4}$
Do	1903	do	6–24	do	.624	$.1\times10^{-4}$
Do	1903	do	0–6	Dried blood, acid phosphate.	.614	$.1\times10^{-4}$
Do	1903	do	6–24	do	.609	$.1\times10^{-4}$
Do	1903	do	0–6	Dried blood, acid phosphate, potassium sulphate.	.600	$.2\times10^{-4}$
Do	1903	do	6–24	do	.592	$.3\times10^{-4}$
Do	1903	do	0–6	Dried blood, acid phosphate, potassium sulphate, lime.	.712	$.3\times10^{-6}$
Do	1903	do	6–24	do	.716	$.2\times10^{-6}$
Do	1903	do	0–6	Lime	.726	$.1\times10^{-6}$
Do	1903	do	6–24	do	.744	$.8\times10^{-7}$
Do	1903	do	0–6	Untreated	.638	$.5\times10^{-5}$
Do	1903	do	6–24	do	.628	$.8\times10^{-5}$

The results from the plots to which acid phosphate has been added do not show any greater H-ion concentration than the ones used as controls. These plots have received rather heavy annual applications of this fertilizer for the past 15 years, the total amount applied being over 3,000 pounds per acre. The fine sandy loam may be some exception. In this case the readings are so nearly the same as from those plots which have received no fertilizer that the differences are within the range of experimental error. Indeed there is as much evidence in indicating an increased basicity from the use of acid phosphate in the clay loam and loam as from an increase in acidity in the sandy loam. This is in agreement with the more recent work of Conner (*5*), Brooks (*4*), and Bear and Salter (*2*).

Additions of lime alone or lime in combinations with the fertilizer materials have materially reduced the acidity in all plots. This is often more marked in the subsoil than in the surface. With the exception of the Cecil clay loam, lime has not been used in sufficient amounts to produce basicity.

EFFECT OF AMMONIUM SULPHATE AND MONOCALCIUM PHOSPHATE ON H-ION CONCENTRATION OF SOIL-FILM WATER

The results heretofore reported in this paper with ammonium sulphate and acid phosphate have been derived from soil suspensions. The question arises, Is the film water of the soil affected in the same or different manner from the free water? To secure data on this question three soils were treated with the ammonium sulphate and monocalcium phosphate at optimum moisture conditions and extractions made with the Morgan apparatus. The materials were applied in solution as a fine spray over the soils in order to get as good distribution as possible. The soils were well worked after the additions to get a uniform mass. Monocalcium phosphate was substituted for acid phosphate on account of its complete solubility. Table VII gives the data derived from treatment with ammonium sulphate.

The film water is shown to be more strongly acid from the treatment with ammonium sulphate than that developed when the same amount of salt is applied in suspension. The indications from this are that methods for estimating soil reaction or lime requirement based on treating the soil with a neutral solution do not give the total acidity in the filtered extract.

The mechanism of this reaction has been the subject of much contention. The explanation which has been offered that the basic radicle has been absorbed by celloidal material; and the acidity developed from the combination of SO_4 with 2H of the slightly ionized H_2O leaves an unbalanced equation. Parker (*10*) contends that the fine soil particles catalyze the reaction $(NH_4)_2SO_4 + 2HOH = 2NH_4OH + H_2SO_4$ with the removal of the entire base from solution by selective adsorption

phenomena. The contention that the base has been removed by combination with the difficultly soluble acids more nearly agrees with the results obtained. The measurements showing the effect of monocalcium phosphate on the H-ion concentration of soil-film water are given in Table VIII.

TABLE VII.—*Effect of ammonium sulphate on the H-ion concentration of soil-film water*

Soil type.	Film water.							Free water.					
	Quantity of soil.	Quantity of ammonium sulphate.	Moisture content, dry basis.	Moisture recovered.	Volume of film water.	Voltmeter readings.	H-ion concentration.	Quantity of soil.	Quantity of ammonium sulphate.	Quantity of water.	Suspension of untreated soil.	Voltmeter readings.	H-ion concentration.
	Lbs.	*Gm.*	*P. ct.*	*P. ct.*	*C. c.*		*Gram-molecules per liter.*	*Gm.*	*Gm.*	*C. c.*	*C. c.*		*Gram-molecules per liter.*
Cecil clay loam	10	10	25	6.0	50	0.523	0.5×10^{-3}	5	0.11	50	50	0.644	0.4×10^{-5}
Do	10	20	25	4.8	50	.518	$.7\times10^{-3}$	5	.22	50	50	.612	$.1\times10^{-4}$
Do	10	30	25	5.4	50	.496	$.1\times10^{-2}$	5	.33	50	50	.591	$.3\times10^{-4}$
Do	.....	.....	.....	.....	.....	.....	.....	5	.....	50	50	.674	$.1\times10^{-5}$
Porter's loam	10	10	25	13.6	50	.532	$.4\times10^{-3}$	5	.11	50	50	.602	$.2\times10^{-4}$
Do	10	20	25	14.1	50	.503	$.1\times10^{-2}$	5	.22	50	50	.588	$.4\times10^{-4}$
Do	10	30	25	11.2	50	.461	$.6\times10^{-2}$	5	.33	50	50	.572	$.8\times10^{-4}$
Do	.....	.....	.....	.....	.....	.....	.....	5	.....	50	50	.686	$.9\times10^{-6}$
Norfolk fine sandy loam	10	10	20	10.6	50	.540	$.2\times10^{-3}$	5	.11	50	50	.584	$.5\times10^{-4}$
Do	10	20	20	11.9	50	.528	$.4\times10^{-3}$	5	.22	50	50	.578	$.6\times10^{-4}$
Do	10	30	20	13.2	50	.509	1.0×10^{-3}	5	.33	50	50	.566	$.1\times10^{-3}$
Do	.....	.....	.....	.....	.....	.....	.....	5	.....	50	50	.670	$.1\times10^{-5}$

TABLE VIII.—*Effect of monocalcium phosphate on the H-ion concentration of soil-film water*

Soil type.	Quantity of soil.	Quantity of monocalcium phosphate.	Moisture content, dry basis.	Moisture recovered.	Volume of film-water.	Voltmeter readings.	H-ion concentration.
	Pounds.	*Gm.*	*Per cent.*	*Per cent.*	*C. c.*		*Gram-molecules per liter.*
Cecil clay loam	10	10	25	7.4	50	0.588	0.4×10^{-4}
Do	10	20	25	6.3	50	.596	$.3\times10^{-4}$
Do	10	30	25	6.1	50	.562	$.3\times10^{-2}$
Porter's loam	10	10	25	11.8	50	.598	$.2\times10^{-4}$
Do	10	20	25	10.9	50	.602	$.2\times10^{-4}$
Do	10	30	25	14.4	50	.562	$.1\times10^{-3}$
Norfolk fine sandy loam	10	10	20	12.2	50	.602	$.2\times10^{-4}$
Do	10	20	20	11.1	50	.591	$.3\times10^{-4}$
Do	10	30	20	11.9	50	.588	$.4\times10^{-4}$

By comparing the data of Table II with those derived from this experiment it is apparent that only excessive applications of monocalcium phosphate have increased the H-ion concentration. The 20-gm. applications of the salt do not show any increase in "true acidity" with any soil used. The clay loam and loam give a higher H-ion concentration

with the 30-gm. addition. The fine sandy loam shows more "true acidity" with a 20-gm. application than when 10 gm. are added, and still more when 30 gm. have been applied.

The fixation or removal from solution of phosphates is supposed to be done by the bases, such as iron, aluminum, calcium, etc., in the soil. The clay loam and loam soils are well supplied with very fine particles of iron and aluminum compounds. They therefore have the capacity of fixing more soluble phosphate than the fine sandy loam, which has a relatively low content of bases. These data are in accord with those obtained by Conner on soils of Indiana.

SUMMARY

The hydrogen electrode has been used for indicating soil reaction on a number of untreated soils in suspension. The soils experimented with represent a wide range in texture of those common to the area of the southeastern portion of the United States, extending from and including the Appalachian Mountains to the Atlantic Ocean. The H-ion concentration varies from almost "true neutrality" to rather excessive "true acidity" in the soils.

With the Morgan apparatus for extracting film water from soils, it is shown that its reaction is the same as the free water, differing only in intensity.

The effects of certain fertilizers on the H-ion concentration of long-time-treated plots of three soils have been measured, with the following results: (1) Ammonia sulphate has materially increased the H-ion concentration of all plots which have received applications of this material. The acidity thus developed extends often to the subsoil. (2) Sodium nitrate has slightly reduced the acidity of the plots to which it has been applied. (3) Potassium sulphate increases the "true acidity" when applied to soils, though not as greatly as ammonium sulphate. (4) Acid phosphate does not appear to have affected in either direction the H-ion concentrations of field soils. (5) Lime materially increases the OH-ion concentration of field plots to which it has been added.

The acidity developed from ammonium sulphate is more intense in the film than in the free water of three soils.

Monocalcium phosphate does not change in any way the soil-film water until excessive amounts are added.

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PURE CULTURES OF WOOD-ROTTING FUNGI ON ARTIFICIAL MEDIA

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INTRODUCTION

The study of wood-rotting fungi by means of cultures on artificial media has been very meager in the past compared to the almost universal use of cultural methods by bacteriologists and workers with strictly parasitic fungi. A critical study of the existing literature on cultures of wood-rotting fungi develops the fact that much of this work was either not done under proper control conditions where the purity of the organism under investigation was guaranteed or the media used by many of the workers consisted of pieces of wood, bread, dung decoctions, etc., and not artificial media of such a character that others could reproduce the media, growth conditions, etc., and thus repeat and verify the experiments.

Brefeld (*1-3*)[2], Falck (*5-7*), Humphrey and Fleming (*8*), Lyman (*11*), Rumbold (*12*), and Zeller (*13, 14*) are some of the workers who have made cultural studies of a number of hymenomycetous fungi on a rather extensive scale. However, the line of investigation followed by most of them has been more along the lines of polymorphism in spore forms, enzymic action, the rot caused by each fungus, and the prevention or control of these fungi in the rotting of structural timber rather than a critical study of their cultural characters on artificial media. Probably the most serious drawback to investigators in working with wood-rotting fungi, especially the Polyporaceae, has been the fact that it was not possible under conditions used by them to obtain with any degree of certainty the sporophores of the various fungi on artificial media.

This paper deals with two lines of investigation of fungous activity when grown in pure cultures: (1) A method by which various wood-rotting fungi can be differentiated from each other by their cultural characters alone when grown upon artificial media; and (2) a method by which the

[1] The writers are under obligations to Dr. E. A. Burt for assistance in identifying the various species of the Thelephoraceae, and to Dr. W. A. Murrill for identifying the more difficult species of the Polyporaceae discussed in this paper.

[2] Reference is made by number (italic) to "Literature cited," pp. 81-82.

fruiting bodies or sporophores of wood-rotting fungi can be produced from pure cultures on artificial media. In this paper the writers have only given in a general way the results of somewhat extended investigations on many species of wood-rotting fungi, leaving for a later article a large amount of detail and the discussion of special methods and culture media which they have found very valuable in working with this group of organisms.

GENERAL METHODS OF EXPERIMENTATION

ORIGIN OF CULTURES

The initial cultures of all of the wood-rotting fungi under investigation by the writers have been obtained from the three following sources: (1) Small pieces of diseased wood, (2) small pieces of sporophores, and (3) spores. Pieces of inoculum 4 or 5 mm. in diameter have been found to be better than smaller pieces. The old idea that the smaller the piece the freer it is from contamination is good reasoning theoretically, but in actual practice pieces of the size mentioned above have been found more viable than small bits and as free from contamination. The larger the piece the greater are the chances of viable mycelium being present. The pieces should be inserted endwise into the middle of the agar slant until about one-half of the wood is buried in the agar. Care should be taken to avoid, as far as possible, burying the wood in the agar, since the covering of agar excludes the air and either retards or prevents entirely the fungus from starting on the culture medium. A pair of long-handled scissors or forceps are especially suitable for this work.

In initial cultures the writers have found it very convenient to use a series of 10 tubes, including 2 tubes each of carrot, malt, cornmeal, prune, and parsnip agars. These agars have been found to give a fairly good growth of mycelium, and at the same time indications of the fruiting, cultural characters, etc. of the organism may be obtained even from these initial cultures.

The writers have made approximately 10,000 cultures of wood-rotting fungi in their preliminary studies here reported. All of the inoculations, both initial and subcultures, have been made in an open room without the use of any special inoculating chamber. The percentage of pure subcultures obtained when the original tube was uncontaminated has been very high. For instance, out of 1,000 transfers recently made only 7 contaminated tubes were found.

METHODS USED IN MAKING SUBCULTURES

The writers desire to describe here a method which they have found very useful in making transfers of fungus cultures when 10 or more transfers are to be made from the same tube. The instruments used in these transfers are a pair of long-handled scissors made by lengthening the handles of a pair of dissecting scissors, a small square glass jar with a

triangular section cut out of the aluminum screw top large enough to hold the plug from the mother tube, and a salt-mouth bottle (holding 300 or 400 c. c.) filled about two-thirds full with 95 per cent alcohol. The blades of the scissors used in making the transfers are kept in this 95 per cent alcohol when not in actual use. The glass jar and top are cleaned by washing in hot water and then dried before using. The opening in the top is thoroughly flamed over an alcohol lamp or Bunsen burner and the jar is placed on its side with the triangular opening toward the operator. During the actual inoculation the cotton plug from the mother tube is placed in the triangular opening with the lower end of the plug inside the jar in such a manner that only the sharp edges of the top of the jar come in contact with the cotton plug. In this position the plug is protected from outside contamination and at the same time the hands of the operator are left free to handle the scissors, two culture tubes, and the cotton plug from the tube to which the transfer is being made.

In making the transfers of certain standardized series it was necessary to obtain small inocula as near the same size for each transfer as possible. The ordinary inoculating needles and loops made either of platinum or of iridio-platinum are too soft and in other ways unsuited for making transfers of fungus mycelium. The writers therefore adopted the use of the scissors for such work, since by using them the mycelial layer on the surface of the agar in the culture tubes can be readily cut and any desired size of inoculum transferred without loss of time and with a minimum of outside contamination.

VEGETATIVE CULTURAL CHARACTERS ON ARTIFICIAL MEDIA

MEDIA USED

In studying the cultural characters of the various fungi as outlined under No. 1 of the introduction, the following general system was adopted: A series of 10 different culture media in agar was used for each fungus. These 10 media were (1) 1.5 and 2 per cent carrot agar, +3.5 to +5.0; (2) 1.5 and 2 per cent malt agar, +7.0; (3) 1.5 per cent beet agar, +2.5 and +3.0; (4) 1.5 per cent celery agar, +9.5 to +15.5; (5) 1.5 per cent bean agar, +1.0 to +1.5; (6) 1.5 and 2 per cent corn-meal agar, +0.25; (7) 1.5 and 2 per cent prune agar, +1.0 and +1.5; (8) 1.5 and 2 per cent alfalfa agar, +13.5 to +15.5; (9) 1.5 per cent parsnip agar, +9.0 to +13.5; and (10) 1.5 and 2 per cent potato agar, +2.0 and +3.5. The acidity of the media here given is based on Fuller's scale and is the actual acidity of the media after tubing and as used in the cultures.

In any series of a given fungus each corresponding agar for each strain had the same percentage and the same acidity. For instance, there were nine strains of *Trametes pini* compared. The carrot agar used for each of these nine sets was 2 per cent and had an acidity of +3.5.

The writers selected the 10 media for the study of the cultural characters of the different fungi not with a view to obtaining vigorous growth but

to get media on which the growth on each would be different for the same organism. In other words, the media used were not intended to develop general characters but specific ones which might differentiate the fungus under investigation from other closely related species.

NUMBER OF TUBES OF EACH MEDIUM INOCULATED

One tube of each of these 10 media was used in the series for any given fungus. Better results would probably have been obtained by using three or more tubes of each medium rather than one, but the writers could not do this in their preliminary work for lack of sufficient equipment. However, in a great majority of cases it is believed that accurate results were obtained with these series of 10, since many of them have been repeated to the fourth and fifth subcultures with different batches of media, and the resulting characters when grown under the conditions described below were practically identical for each subculture of the fungus for each medium.

POSITION OF CULTURE TUBES IN REFERENCE TO GRAVITY

After inoculating the series of 10 tubes, they were placed in a horizontal position, side by side, in shallow boxes with the surface of the agar slant uppermost. The boxes were from 2 to 4 cm. deep and about 14 to 14.5 cm. wide. The culture tubes (150 mm. long or longer) had their tops resting on the upper edges of the boxes and were therefore tilted at a slight angle. These boxes were then placed on shelves in front of windows with a western exposure where they received all of the diffused light which came through and during the afternoon received the direct rays of the sun from one to four hours daily. Under these conditions the agar in the tubes gradually dried and the upper portion of it separated from the glass, leaving a space of varying depth between the agar and the glass, on which the aerial mycelium could grow even to the bottom of the tube.

AMOUNT OF DIRECT SUNLIGHT CULTURES RECEIVED

The earlier cultures of the writers received only one or two hours of direct sunlight. As the season advanced, the quantity of direct sunlight received became greater, until finally the amount received was judged to be too great and the intensity of the direct sunlight was decreased, first, by a single screen of cheesecloth tacked over the front of the frames holding the culture tubes. Later, a second piece of cheesecloth was tacked over the first one. The general effect of the sunlight on the cultures in the tubes thus exposed was to check the growth of the fungi, compared to similar tubes when placed in very weak, diffused light or absolute darkness. The sunlight also seems to intensify the colors of the aerial mycelium when it is normally other than white.

TEMPERATURE RECORDS

During the entire time the cultures were under observation, two thermographs were run continuously. One was placed on the shelf with the fungi exposed to sunlight. A soil thermograph was used to record the temperature of the tubes kept in the dark.

DEFINITION OF TERMS USED

It was found early in the study of the cultural characters of the fungi under consideration that a set of descriptive terms especially adapted to the cultures of fungi grown under the conditions here described would have to be used. The terms employed in the tables in this paper and in the body of the text are those usually found in ordinary botanical literature, but they have been modified somewhat to fit the conditions obtaining for fungus growth. The fungus growth on artificial media is divided by the writers into two general classes, aerial and submerged. The aerial mycelium consists of that which is on or above the surface of the agar; the submerged mycelium includes all that is beneath the surface of the agar.

Great difficulty was found in obtaining appropriate terms which would express the character of growth of the aerial mycelium. In describing this aerial growth terms which are usually used in describing the pubescence of leaf surfaces have been employed. In other words, the surface of the agar is considered as the surface of a leaf and the character of the mycelium growing on this surface is discussed in terms of leaf pubescence with some minor modifications made necessary by the character of the organism under discussion. The following terms as used by the writers require special definition, since they depart in some instances from the usually accepted definitions of these terms:

Appressed: Mycelium which is prostrate on the surface of the agar. This with many fungi is the first stage in the aerial growth of the mycelium. Later this appressed mycelium may give place to other forms.

Cobwebby: Long, weak, intertangled hairs which are not thick enough to be either woolly or felty and are not short enough to be considered as downy.

Cottony: Erect, rather long (3 to 5 mm.) mycelium spreading in all directions.

Downy: Short, fine hairs, loosely scattered over the surface of the mycelium, giving it a downy appearance.

Felty: Matted with intertwined hairs, resembling felt.

Floccose: Scattered patches of short mycelium.

Plumose: Tufts of mycelium with a central axis from which short hyphae radiate.

Silky: Long parallel threads of mycelium, more or less prostrate, like combed silk.

Sodden: Mycelium having a water-soaked appearance; usually such mycelium is appressed.

Subfelty: A thin layer of mycelium consisting of short intertwined hairs.

Velvety: Layer of mycelium with distinct, dense, straight, short hairs like pile of velvet.

Woolly: A dense mass of mycelium consisting of long, tortuous, matted hairs. Cottony and woolly may both later become felty by the long hairs becoming matted and prostrate.

Hyphenated compound words, like "appressed-downy," "felty-woolly," indicate a condition intermediate between the two names, while "downy to appressed" means that the older portions are downy, while the younger portions are appressed.

In all of the tubes the growth of the fungus is both lateral and longitudinal. Of course, the lateral extension is very limited, since the inside diameter of the tubes is only about 20 mm., while the length varies with the length of the agar in the tube. The first record for growth shown in all the tables is always that of the lateral growth. For instance, a record showing 20 by 30 mm. means that the lateral growth was 20 mm. and the longitudinal 30 mm. When the same set of figures are repeated for two intervals of time, like 60 days, 20 by 80 mm., and 80 days, 20 by 80 mm., this indicates that the growth of the fungus had reached the bottom of the tube at the first record given and would therefore be the same for the second interval of time.

IMPORTANT DIFFERENTIAL CRITERIA

The following criteria have been found of value in the differentiation of the various species: (1) Macroscopic characters, including rapidity of growth, color of aerial and submerged mycelium, character of aerial mycelium such as to texture, etc., staining of the agar, decoloration of the agar, the comparative rate of growth between the aerial and submerged mycelium, especially when the submerged mycelium is colored and markedly in advance of the aerial; (2) microscopic characters, such as septation, branching, size and color of hyphæ, clamp connections, polymorphism in spore formation, etc. A few of the species of Polyporaceae examined by the writers have in addition to the usual basidiospores other spore forms variously known as conidia, oidia, chlamydospores, etc. These various nonbasidiosporic forms may be divided into two general groups, spores which are borne on the aerial hyphae and the so-called spores which are borne on the submerged hyphae, often referred to as chlamydospores. The latter have been found by the writers to be more widely distributed in the Polyporaceae than the aerial spores and their presence and characters as well as those of the aerial should always be noted, since they are of great diagnostic value.

It will be noted from the tables that certain fungi have colorless or white aerial mycelium throughout on certain agars, while others have colored depending upon the agar used. Such color differences are very important, since they are usually constant for a given species on a given agar.

In some species of the fungi examined the submerged mycelium in certain media is constantly colored, while in other species, whether the submerged mycelium is colored or colorless, seems to depend upon certain environmental factors, such as the amount of moisture present in the medium or the acidity or alkalinity of the medium.

Some of the most important criteria for distinguishing different but closely related fungi are found in the first 10 or 15 days of the growth of the subcultures, such as rapidity of growth, color changes in the mycelium, staining of the agar, decoloration of media, etc. Important characters which are sharply defined at one stage of growth often disappear or are obscured by the later mycelial development; and for this reason the cultural data in the tables have been given for several periods of time in the growth of the cultures, say at 10, 20, and 30 day intervals.

INFLUENCE OF SUNLIGHT ON CULTURAL CHARACTERS

One of the special benefits which seems to be derived from exposing cultures to the sunlight is the accentuating of the color characteristics and toning down of the mycelial growth of the fungus, thereby making it more characteristic and uniform for a given species than when placed under similar conditions in the darkness.

The differentiation of the characters of the mycelium produced, both as to texture and color of the aerial mycelium, is very much more marked when the cultures are grown in the presence of light at ordinary room temperatures than when grown in incubators at the optimum and constant temperature for the mycelial growth of the fungus under consideration. This probably explains why no one up to the present time has seriously attempted to differentiate the various species of wood-rotting fungi by means of cultural characteristics alone.

Furthermore, the cultures when grown in darkness and at a more or less constant and high temperature overrun very rapidly the surface of the agar in the tube, thus obscuring the real growth of the fungus as observed in the cultures subject to daylight conditions.

GROWTH OF WOOD-ROTTING FUNGI ON AGARS

TEXTURE.—In the growth of wood-rotting fungi on agars the fungus as it spreads from the inoculum on to the surface of the slant proper assumes certain well-defined stages in its growth, which may be roughly divided into two general divisions: (1) Fungi whose advancing young mycelial zone is appressed and (2) fungi whose advancing zone is downy, felty, woolly, etc. There is but little real difference between these two methods of growth, since as a rule the character of the mycelium first to appear is appressed. If the true aerial mycelium, in contradistinction to that which is strictly prostrate on the surface of the agar keeps pace in its growth with the appressed mycelium, the zone of growth will be downy, felty, woolly, etc. If, on the other hand, the growth of the strictly aerial mycelium is much retarded, the appressed mycelium will present a well-defined zone from one to several millimeters across.

The appressed mycelium is usually either colorless or both colorless and sodden, and from this the true aerial mycelium usually develops. The cottony mycelium as a rule does not persist in this condition for

any great length of time. The long, divergent, aerial strands usually become more or less compact and finally felty. The usual steps in the growth of the mycelium of a fungus are first appressed, then downy, then felty, woolly, etc. Cottony mycelium usually develops the cottony stage immediately from the appressed condition. Many of the fungi pass so rapidly from the downy to the felty or woolly stage that it is unnecessary in the description to indicate that there is an intermediate downy stage.

Colors.—The colors of the fungus as a rule follow certain definite changes. Excluding the color of the mycelium on the inoculum, the first color which usually appears in the early stages of the fungus will be either colorless or white, depending to a considerable extent on whether the young mycelium is appressed or downy. The next step in the color changes will be for the older whitish areas to become light buff[1], warm buff, antimony yellow, etc., if the fungus happens to belong to some of the brown polyporaceae. As the culture ages, the color of the mycelium on the older areas will assume a deeper and deeper tone until finally a color is reached beyond which no appreciable change is observed. In the large majority of cases the color of the older mycelium constitutes a rather extended area compared to that of the younger zone. In practically every instance the cultures obtained from any given fungus on at least several of the culture media will approach very closely the color of the sporophores as they appear in nature. For instance, if one is attempting to grow cultures of *Polyporus dryophilus*, *Fomes texanus*, or other brown fungi, one would expect to have at least several of the culture tubes with brown mycelium similar to that of the fungus.

TABLES SHOWING CULTURAL CHARACTERS

In this preliminary report only a few tables are given out of a large number which the writers have complied on the cultural characters of certain species of wood-rotting fungi. The following species having brown sporophores are given to illustrate the close resemblance in colors, texture, etc., of the same fungus on different hosts: Four strains of *Fomes texanus* (Tables I–IV) and two strains of *Polyporus farlowii* (Tables V–VI).

[1] The colors used in this paper are according to the following standards:
Ridgway, Robert. Color standards and color nomenclature. 43 p., 53 col. pl. Washington, D. C., 1912.

TABLE I.—*Cultural data for Fomes texanus from Juniperus monosperma (FP 21653), New Mexico.*

[Subculture 4 from mycelium on corn-meal agar; initial culture from tissue. Series inoculated on Mar. 12, 1917]

Medium (agar)	Color of aerial mycelium.				Color of submerged mycelium.			
	11 days.	22 days.	38 days.	60 days.	11 days.	22 days.	38 days.	60 days.
Carrot	....	Ochraceous tawny	Argus brown to antimony yellow.	Argus brown to buckthorn brown.	....	Brownish	Blackish brown	Blackish brown.
Malt	Warm buff	Antimony yellow	Amber brown to antimony yellow.	Buckthorn brown to antimony yellow.	Colorless	Chestnut	Liver brown	Do.
Beet	....	Cinnamon brown to antimony yellow.	Chestnut	Chestnut	....do	Blackish brown	Blackish brown	Blackish brown.
Celery	....	Buckthorn brown to colorless.	Argus brown to sodden.	Argus brown to sodden.	....	Auburn	....do	Do.
Bean	Colorless	Colorless	Colorless	Colorless	Colorless	Russet	Chestnut	Chestnut.
Corn meal	Russet to colorless.	Russet to colorless.	Chestnut to colorless.	Amber to colorless.	Russet	Mummy brown	Liver brown	Mars brown.
Prune	Warm buff	Cinnamon brown to yellow ocher.	Chestnut	Chestnut to amber brown.	Colorless	....do	Chestnut	Blackish brown.
Alfalfa	Buckthorn brown	Sudan brown	Argus brown to buckthorn brown.	Argus brown	....do	Prout's brown	Russet	....
Parsnip	....	Russet to cinnamon brown.	Amber brown to antimony yellow.	Chestnut	....	Mummy brown	Blackish brown	Blackish brown.
Potato	Light buff	Ochraceous tawny to antimony yellow.	Sayal brown	Sayal brown	Colorless	....do	Cinnamon brown	Do.

Medium (agar).	Texture of aerial mycelium.				Size of area.			
	11 days.	22 days.	38 days.	60 days.	11 days.	22 days.	38 days.	60 days.
					Mm.	*Mm.*	*Mm.*	*Mm.*
Carrot	....	Velvety	Velvety	Velvety	....	20×22	20×30	20×45
Malt	Velvety	....do	....do	....do	4×6	20×25	20×55	20×70
Beet	....	....do	....do	....do	....	15×28	18×35	20×45
Celery	....	Velvety to appressed	Velvety to appressed	Velvety to appressed	....	15×32	20×35	20×42
Bean	Appressed	Appressed	Appressed	Appressed	4×1	10×12	20×25	20×35
Corn meal	Velvety to appresed	Velvety to appressed	Velvety to appressed	Velvety to appressed	2×3	18×20	20×40	20×60
Prune	Velvety	Velvety	Velvety	Velvety	2×3	20×25	20×40	20×65
Alfalfa	....do	....do	....do	....do	12×14	20×35	20×70	20×70
Parsnip	....	....do	....do	....do	....	15×25	20×32	20×45
Potato	Velvety	....do	....do	....do	1×2	15×18	15×20	20×30

TABLE II.—*Cultural data for Fomes texanus from Juniperus monosperma (FP 21807), New Mexico.*

[Subculture 2 from mycelium on alfalfa agar; initial culture from tissue. Series inoculated on Mar. 12, 1917]

Medium (agar).	Color of aerial mycelium.				Color of submerged mycelium.			
	11 days.	23 days.	38 days.	60 days.	11 days.	23 days.	38 days.	60 days.
Carrot		Ochraceous tawny	Argus brown	Argus brown		Brownish	Liver brown	Blackish brown.
Malt	Warm buff	Buckthorn brown to antimony yellow.	Argus brown to buckthorn brown.	Chestnut to buckthorn brown to antimony yellow.	Colorless	Mummy brown	do	Chestnut.
Beet		Cinnamon brown to antimony yellow.	Chestnut to sodden.	Chestnut	do	Blackish brown	Blackish brown	Blackish brown.
Celery		do	Chestnut	Argus brown to sodden.	do	Mummy brown	do	Do.
Bean	Colorless	Colorless	Colorless	Colorless	do	Russet	Russet	Chestnut.
Cornmeal	Warm buff	Ochraceous tawny to russet.	Chestnut to colorless.	Amber brown	Russet	Mummy brown	Liver brown	Mars brown.
Prune	do	Cinnamon brown to yellow ocher.	Chestnut	do	Colorless	do	do	Blackish brown.
Alfalfa	Buckthorn brown.	Buckthorn brown.	Sudan brown	Argus brown to buckthorn brown.	do	Prout's brown	Prout's brown	Russet.
Parsnip		Russet to cinnamon brown.	Chestnut	Chestnut	do	Mummy brown	Chestnut	Blackish brown.
Potato	Warm buff	Ochraceous tawny	Sayal brown	Sayal brown	Russet	Mummy brown	Blackish brown	Do.

Medium (agar).	Texture of aerial mycelium.				Size of area.			
	11 days.	22 days.	38 days.	60 days.	11 days.	23 days.	38 days.	60 days.
					Mm.	*Mm.*	*Mm.*	*Mm.*
Carrot		Velvety	Velvety	Velvety		15×18	15×20	20×36
Malt	Velvety	do	do	do	5×7	20×35	20×40	20×65
Beet	do	do	do	do		15×25	18×30	20×35
Celery	do	do	do	do		18×20	20×25	20×32
Bean	Appressed	Appressed	Appressed	Appressed	1×2	10×18	10×20	20×30
Cornmeal	do	Velvety to appressed	Velvety to appressed	Velvety	1×2	18×30	20×35	20×55
Prune	Velvety	Velvety	Velvety	Velvety	5×7	20×30	20×40	20×60
Alfalfa	do	do	do	do	2×3	20×40	20×45	20×70
Parsnip		do	do	do		18×22	20×30	20×50
Potato	Velvety	do	do	do	2×3	10×10	10×12	12×15

TABLE III.—*Cultural data for Fomes texanus from Juniperus scopulorum (FP 21806), New Mexico*

[Subculture 2 from mycelium on alfalfa agar; initial culture from tissue. Series inoculated on Mar. 12, 1917]

Medium (agar)	Color of aerial mycelium.				Color of submerged mycelium.			
	11 days.	22 days.	38 days.	70 days.	11 days.	22 days.	38 days.	70 days.
Carrot		Ochraceous tawny.	Amber brown	Amber brown to buckthorn brown.		Mummy brown	Liver brown	Blackish brown.
Malt	Warm buff	do	Chestnut to antimony yellow.	Chestnut to antimony yellow.		do	do	Do.
Beet	Light buff	Cinnamon brown to antimony yellow.	Chestnut to sodden.	Buckthorn brown to antimony yellow.		Blackish brown	Blackish brown	Blackish brown.
Celery	Buckthorn brown	Antique brown to antimony yellow.	Argus brown	Argus brown		Auburn	do	Do.
Bean	Colorless	Colorless	Colorless	Colorless		Russet	Russet	Chestnut.
Corn meal	Light buff	Light buff to colorless.	Amber brown to colorless.	Amber brown	Russet	Mummy brown	Liver brown	Mars brown.
Prune	Warm buff	Cinnamon brown to yellow ocher.	Chestnut to yellow ocher.	Chestnut to amber brown.	Colorless	do	do	Blackish brown.
Alfalfa	Buckthorn brown	Buckthorn brown to cinnamon brown.	Sudan brown	Argus brown	do	Prout's brown	Prout's brown	Mummy brown.
Parsnip		Russet to cinnamon brown.	Amber brown	Amber brown	do	Mummy brown	Chestnut	Blackish brown.
Potato	Light buff	Ochraceous tawny.	Sayal brown	Sayal brown	Russet	do	do	Do.

Medium (agar).	Texture of aerial mycelium.				Size of area.			
	11 days.	22 days.	38 days.	70 days.	11 days.	22 days.	38 days.	70 days.
					Mm.	*Mm.*	*Mm.*	*Mm.*
Carrot		Velvety	Velvety	Velvety		15×25	20×38	20×72
Malt	Velvety	do	do	do	4×5	20×30	20×45	20×62
Beet	do	do	do	do	2×1	15×15	18×20	20×45
Celery	do	do	do	do	10×20	20×25	20×32	20×40
Bean	Appressed	Appressed	Appressed	Appressed	3×4	8×8	15×25	20×40
Corn meal	do	Velvety to appressed	Velvety to appressed	Velvety	1×2	20×20	20×40	20×60
Prune	Velvety	Velvety	Velvety	do	3×5	20×25	20×38	20×60
Alfalfa	do	do	do	do	3×4	20×30	20×40	20×65
Parsnip	do	do	do	do		15×20	20×35	20×55
Potato	Appressed velvety	Appressed velvety	Appressed velvety	Velvety	1×2	8×8	8×16	20×35

TABLE IV.—*Cultural data for Fomes texanus from Juniperus scopulorum (FP 21760), New Mexico*

[Subculture 3 from mycelium on corn meal agar; initial culture from tissue. Series inoculated on Mar. 12, 1917]

Medium (agar).	Color of aerial mycelium.				Color of submerged mycelium.			
	11 days.	22 days.	38 days.	60 days.	11 days.	22 days.	38 days.	60 days.
Carrot		Cinnamon brown.	Amber brown	Amber brown to buckthorn brown.		Mummy brown	Liver brown	Blackish brown.
Malt	Warm buff	Buckthorn brown.	Buckthorn brown to antimony yellow.	Buckthorn brown to antimony yellow.	Colorless	Chestnut	do	Do.
Beet		Prout's brown to cinnamon brown.	Chestnut	Chestnut		Blackish brown	Blackish brown	Do.
Celery		Warm buff	Sudan brown	Argus brown		Mummy brown	do	Do.
Bean	Colorless	Colorless	Colorless	Colorless	Colorless	Russet	Russet	Chestnut.
Corn meal	do	Russet to colorless.	Chestnut to colorless.	Amber brown to colorless.	Russet	Mummy brown	Liver brown	Mars brown.
Prune	Warm buff	Cinnamon brown to yellow ocher.	Chestnut to Sandford brown.	Chestnut to amber brown.	Colorless	Blackish brown	Blackish brown	Blackish brown.
Alfalfa	Buckthorn brown.	Buckthorn brown.	Sudan brown	Argus brown	do	Prout's brown	Prout's brown	Mummy brown.
Parsnip		Russet to cinnamon brown.	Chestnut	Amber brown to buckthorn brown.		Mummy brown	Chestnut	Blackish brown.
Potato		Ochraceous tawny.	Sayal brown	Sayal brown		Blackish brown	Blackish brown	Do.

Medium (agar).	Texture of aerial mycelium.				Size of area.			
	11 days.	22 days.	38 days.	60 days.	11 days.	22 days.	38 days.	60 days.
					Mm.	*Mm.*	*Mm.*	*Mm.*
Carrot		Velvety	Velvety	Velvety		18×22	20×28	20×42
Malt	Velvety	do	do	do	1×2	20×25	20×40	20×62
Beet		do	do	do		5×10	10×12	10×15
Celery		do	do	do		15×20	20×25	20×35
Bean	Appressed	Appressed	Appressed	Appressed	1×2	10×20	15×25	20×40
Corn meal	do	Appressed to velvety	Appressed to velvety	Appressed to velvety	2×1	10×10	20×40	20×60
Prune	Velvety	Velvety	Velvety	Velvety	2×3	20×30	20×45	20×70
Alfalfa	do	do	do	do	4×6	20×32	20×42	20×62
Parsnip	do	do	do	do		12×20	20×40	20×60
Potato		do	do	do		8×15	10×18	10×22

TABLE V.—*Cultural data for Polyporus farlowii from Populus italica (FP 21402), New Mexico*

[Subculture 6 from mycelium on malt agar; initial culture from tissue]

Medium (agar).	Color of aerial mycelium.				Color of submerged mycelium.			
	4 days.	16 days.	30 days.	63 days.	4 days.	16 days.	30 days.	63 days.
Carrot	Warm buff	Buckthorn brown.	Buckthorn brown.	Buckthorn brown.	Colorless	Colorless	Colorless	Colorless.
Malt		do	do	do	do	do	do	Do.
Beet	Colorless	do	do	do	do	do	do	Do.
Celery	White	do	do	do	do	do	Stained agar Sandford brown.	Stained agar Sandford brown.
Bean	do	White	White	White	do	do	Colorless	Colorless.
Corn meal		Buckthorn brown to yellow ocher.	Buckthorn brown.	Buckthorn brown.		do	do	Do.
Prune		Buckthorn brown.	do	do		do	do	Do.
Alfalfa		White	White	White		do	do	Do.
Parsnip		Buckthorn brown to antimony yellow.	Buckthorn brown.	Buckthorn brown.		do	do	Do.
Potato		Warm buff	Warm buff	Warm buff		do	do	Do.

Medium (agar).	Texture of aerial mycelium.				Size of area.			
	4 days.	16 days.	30 days.	63 days.	4 days.	16 days.	30 days.	63 days.
					Mm.	*Mm.*	*Mm.*	*Mm.*
Carrot	Cottony	Woolly, dense	Woolly, dense	Woolly, dense	1×2	20×85	20×85	20×85
Malt		Woolly	Woolly	do		20×70	20×90	20×90
Beet	Appressed	Cottony	Woolly, dense	do	2×3	20×60	20×85	20×85
Celery	Cottony	Woolly	Woolly	do	4×5	20×80	20×90	20×90
Bean		Appressed to cobwebby	Silky	Silky to downy		20×50	29×75	20×80
Corn meal	Cottony	Velvety to felty	Woolly	Woolly, dense		20×70	20×80	20×80
Prune		do	Felty	Felty		20×50	20×85	20×90
Alfalfa		Cottony to silky dense	Woolly to silky	Felty to silky		20×70	20×90	20×90
Parsnip	Cottony	Dense cottony to woolly	Woolly, dense	Woolly, dense		20×75	20×90	20×90
Potato		Cottony	Cottony	Felty		20×20	20×75	20×75

TABLE VI.—*Cultural data for Polyporus farlowii from Acer negundo (FP 21591), New Mexico*

[Subculture 2 from mycelium on potato agar; initial culture from tissue. Series inoculated on Apr. 7, 1917]

Medium (agar).	Color of aerial mycelium.			Color of submerged mycelium.			Texture of aerial mycelium.			Size of area.		
	4 days.	36 days.	60 days.	4 days.	36 days.	60 days.	4 days.	36 days.	60 days.	4 days.	36 days.	60 days.
										Mm.	*Mm.*	*Mm.*
Carrot	Warm buff	Buckthorn brown.	Buckthorn brown.	Colorless	Colorless	Colorless	Cottony to silky.	Woolly, dense	Woolly, dense	20×20	20×75	20×75
Malt	Antimony yellow.	do	do	do	do	do	Velvety	do	Felty	10×18	20×70	20×80
Beet	Yellow ocher	do	do	do	do	do	Cottony	Woolly to felty	do	10×25	20×60	20×80
Celery	Warm buff	do	do	do	do	do	do	Woolly, dense	do	15×28	20×75	20×75
Bean	Colorless	Colorless	Colorless	do	do	do	Appressed, scant.	Appressed, scant.	Appressed, scant.	10×20	20×65	20×75
Corn meal	Warm buff	Buckthorn brown.	Buckthorn brown.	do	do	do	Cottony	Woolly, dense	Woolly	15×25	20×90	20×90
Prune		do	do	do	do	do		Felty	Felty		20×65	20×65
Alfalfa	White	White	White	do	do	do	Cottony to silky.	Felty to silky	Felty to silky	15×25	20×75	20×75
Parsnip	Antimony yellow.	Buckthorn brown.	Buckthorn brown.	do	do	do	Cottony	Felty	Felty	15×20	20×85	20×85
Potato	Light buff	Warm buff	Warm buff	do	do	do	do	Woolly	do	15×15	20×70	20×70

DISCUSSION OF CULTURAL TABLES

Fomes texanus. The cultural characters for all of the four strains show remarkable uniformity for each of the corresponding agars in the series of ten. This fungus seems to be more susceptible to differences in the amount of sunlight it received than any species thus far investigated. When the intensity of the sunlight was diminished by the cheesecloth screens previously mentioned, this species on several of the agars immediately responded to the decrease in light by making a more vigorous growth and turning a lighter shade of brown.

Polyporus farlowii. This fungus is also very uniform in growth on each of the 10 agars as the tables show. When grown in the dark, the strain from *Acer negundo* developed a submerged mycelium on prune and corn-meal agars which was Mars brown in place of colorless. Whether this change in colors of the mycelium was due to the darkness is doubtful since the other strain from *Populus italica* when grown in darkness still retained its colorless mycelium the same as when grown in the light.

Series were grown of nine strains of *Trametes pini* obtained from material collected in four States and growing on seven hosts—viz, *Pinus echinata*, *P. flexilis*, *P. ponderosa*, *Picea engelmannii*, *Pseudotsuga taxifolia*, *Abies arizonica*, and *A. lasiocarpa.* This series represented subcultures ranging from 1 to 7. While there was some slight variation, especially in the colors of the mycelium of the various strains, the differences were not so marked as to constitute real specific characters. There was practically no difference between the cultural characters obtained from the different subcultures. This would indicate that the general fundamental characters of the fungus are not materially changed through successive subcultures, at least in this instance to the seventh subculture.

As will be noted from the temperature record, there was rather a wide variation in temperature during the time the various series of cultures were growing. Nevertheless the general cultural characters as shown in tables are practically identical. This identity of characters for a given organism on a given culture medium is still more marked when a series of from three to six tubes of the same agar for the same strain is made at the same time and then compared as the growth progresses.

CULTURAL CHARACTERS FOR DIFFERENT STRAINS OF SAME FUNGUS

The sources of the initial cultures from the nine strains of *Trametes pini* were five from sporophore tissue and four from infected wood, representing seven hosts, while the cultures from *Fomes texanus* all were from tissue but from two hosts. The *Polyporus farlowii* cultures were both from tissue but from different hosts. A careful comparison of the cultural characters of the various strains of each of these fungi shows no appreciable differences between cultures of a given fungus whether obtained from infected wood or from sporophores; neither do the hosts

of the fungus seem to make any marked changes in the fundamental cultural characters, as is clearly shown in the various tables when strains from different hosts are compared. There may be minor differences due to the host from which the strain came, but nothing more.

The comparison of the cultural characters of many species of parasitic fungi has long been recognized as a reliable index to the identity of the fungus under investigation, and there is no reason why the cultural characters of wood-rotting fungi which are just as uniform and dependable should not be used for identification purposes.

The writers have purposely avoided going into a discussion of the results of the use of synthetic agars and of other special media, as these will be taken up in a later article. They have presented here the results obtained from agars easily made and apparently of a uniform enough composition for similar cultural characters to appear on different batches of the same agar even when the acidity, alkalinity, and water content vary considerably. Just how great a variation in these factors must occur to produce a decided change in the cultural characters is a problem for future investigation.

EXAMPLES OF THE DIAGNOSTIC VALUE OF CULTURAL CHARACTERS IN SPECIES DETERMINATION

In comparing the cultural characters of closely related but really distinct species marked and constant differences in the character of the mycelium will be found on certain corresponding agars in the series of cultures representing the two species, while if the two fungi are really the same species no constant differences of specific rank will be found. The following fungi will illustrate the diagnostic value of the cultural characters in determining the real position of the species.

TABLE VII.—*Cultural data for Fomes rimosus from Acacia roemeriana (FP 21681), Texas*

[Subculture 3 from mycelium on malt agar; initial culture from tissue. Series inoculated on May 31, 1917]

Medium (agar).	Color of aerial mycelium.				Color of submerged mycelium.			
	10 days.	20 days.	30 days.	40 days.	10 days.	20 days.	30 days.	40 days.
Carrot	Clay color to light buff.	Clay color to warm buff.	Clay color to warm buff.	Clay color	Colorless	Colorless	Colorless	Colorless.
Malt	Clay color to warm buff.	Amber brown to buckthorn brown.	Amber brown to buckthorn brown.	Amber brown	do	Amber brown	Amber brown to colorless.	Amber brown to colorless.
Beet	Sodden	Light buff to sodden	Warm buff to mainly sodden.	Warm buff to mainly sodden.	do	Colorless 11–15 mm. beyond aerial mycelium.	Colorless 20–30 mm. beyond aerial mycelium.	Colorless 40–45 mm. beyond aerial mycelium.
Celery	Warm buff to colorless.	Warm buff to colorless.	Warm buff to mainly colorless.	Warm buff to mainly colorless.	do	Colorless 20–30 mm. beyond aerial mycelium.	Colorless 40–45 mm. beyond aerial mycelium.	Colorless 45–50 mm. beyond aerial mycelium.
Bean	Sodden	Light buff to mainly sodden.	Light buff to mainly sodden.	Light buff to mainly sodden.	do	Colorless 10–12 mm. beyond aerial mycelium.	Colorless 20–30 mm. beyond aerial mycelium.	Colorless 25–32 mm. beyond aerial mycelium.
Corn meal	Clay color to colorless.	Clay color to colorless.	Clay color to colorless.	Clay color to colorless.	do	Colorless	Colorless	Colorless.
Prune	do	Buckthorn brown to warm buff.	Buckthorn brown	Buckthorn brown	do	Tawny to colorless.	Tawny to colorless.	Tawny to colorless.
Alfalfa	Light buff	Maize yellow to colorless.	Maize yellow to colorless.	Warm buff to mainly colorless.	do	Colorless	Colorless	Colorless.
Parsnip	Buckthorn brown to warm buff.	Buckthorn brown to light buff.	Buckthorn brown.	Buckthorn brown.	do	Mars brown to colorless.	Mars brown to colorless.	Mars brown to colorless.
Potato	Warm buff to colorless.	Warm buff to colorless.	Warm buff to colorless.	Warm buff to colorless.	do	Colorless	Colorless	Colorless.

Medium (agar).	Texture of aerial mycelium.				Size of area.			
	10 days.	20 days.	30 days.	40 days.	10 days.	20 days.	30 days.	40 days.
					Mm.	*Mm.*	*Mm.*	*Mm.*
Carrot	Subfelty to silky	Felty	Felty	Felty	20×32	20×58	20×78	20×78
Malt	Subfelty	Felty to downy	Felty to downy	Felty	20×26	20×60	20×75	20×75
Beet	Appressed	Downy to mainly appressed	Downy to mainly appressed	Downy to mainly appressed	15×15	20×40	20×55	20×57
Celery	Subfelty to appressed	Felty to mainly appressed	Felty to mainly appressed	Felty to mainly appressed	10×15	20×45	20×70	20×70
Bean	Appressed	Downy to mainly appressed	Downy to mainly appressed	Downy to mainly appressed	10×15	20×35	20×50	20×55
Corn meal	Downy-felty to appressed	Downy-felty to appressed	Downy-felty to appressed	Downy-felty to appressed	20×25	20×46	20×60	20×75
Prune	do	Subfelty to downy	Felty to downy	Felty to downy	20×38	20×60	20×70	20×75
Alfalfa	Cottony-silky	Silky-felty to appressed	Silky-felty to appressed	Silky-felty to appressed	10×15	20×32	20×45	20×75
Parsnip	Felty	Felty to appressed	Felty to appressed	Felty to appressed	20×30	20×55	20×75	20×82
Potato	Downy to appressed	Downy to appressed	Downy to appressed	Downy to appressed	15×18	20×25	20×26	20×27

TABLE VIII.—*Cultural data for Fomes rimosus from Acacia roemeriana (FP 21817), Texas*

[Subculture 1 from mycelium on prune agar; initial culture from infected wood. Series inoculated on May 31, 1917]

Medium (agar).	Color of aerial mycelium.				Color of submerged mycelium.			
	10 days.	20 days.	30 days.	40 days.	10 days.	20 days.	30 days.	40 days.
Carrot	Antimony yellow to light buff.	Clay color to warm buff.	Clay color to warm buff.	Clay color to warm buff.	Colorless	Colorless	Colorless	Colorless
Malt	Clay color to warm buff.	Buckthorn brown to white.	Amber brown to buckthorn brown.	Amber brown to buckthorn brown.	do	Amber brown	Amber brown to colorless.	Amber brown to colorless.
Beet	Sodden	Warm buff to sodden.	Buckthorn brown to mainly sodden.	Buckthorn brown to mainly sodden.	do	Colorless 10–12 mm. beyond aerial mycelium.	Colorless 15–20 mm. beyond aerial mycelium.	Colorless 10–15 mm. beyond aerial mycelium.
Celery	Warm buff to colorless.	Warm buff to colorless.	Warm buff to colorless.	Warm buff to colorless.	do	Colorless 20–30 mm. beyond aerial mycelium.	Colorless 40 mm. beyond aerial mycelium.	Colorless 30 mm. beyond aerial mycelium.
Bean	Light buff	Light buff to mainly sodden.	Light buff to mainly sodden.	Light buff to mainly sodden.	do	Colorless 20–25 mm. beyond aerial mycelium.	Colorless 30–35 mm. beyond aerial mycelium.	Colorless 25–30 mm. beyond aerial mycelium.
Corn meal	Warm buff to colorless.	Warm buff to colorless.	Warm buff to colorless.	Warm buff to colorless.	do	Colorless	Colorless	Colorless.
Prune	Clay color to colorless.	Buckthorn brown to warm buff.	Buckthorn brown to white.	Buckthorn brown to light buff.	do	Mars brown to colorless.	Mars brown to colorless.	Mars brown to colorless.
Alfalfa	Light buff	Maize yellow to colorless.	Maize yellow to colorless.	Warm buff to mainly colorless.	do	Colorless 10–15 mm. beyond aerial mycelium.	Colorless 20–25 mm. beyond aerial mycelium.	Colorless 25–30 mm. beyond aerial mycelium.
Parsnip	Buckthorn brown to warm buff.	Buckthorn brown to light buff.	Buckthorn brown.	Buckthorn brown.	do	Mars brown to colorless.	Mars brown to colorless.	Mars brown to colorless.
Potato	Light buff to colorless.	Light buff to colorless.	Light buff to colorless.	Light buff to colorless.	do	Colorless	Colorless	Colorless.

Medium (agar).	Texture of aerial mycelium.				Size of area.			
	10 days.	20 days.	30 days.	40 days.	10 days.	20 days.	30 days.	40 days.
					Mm.	*Mm.*	*Mm.*	*Mm.*
Carrot	Subfelty to silky	Felty	Felty	Felty	20×25	20×58	20×75	20×75
Malt	Subfelty to downy	Felty to downy	Felty to downy	do	20×25	20×25	20×65	20×75
Beet	Appressed	Downy to appressed	Downy to mainly appressed	Downy to mainly appressed	12×10	20×22	20×28	20×32
Celery	Subfelty	Felty to mainly appressed	Felty to mainly appressed	Felty to mainly appressed	10×12	20×50	20×70	20×70
Bean	Downy	Downy to mainly appressed	Downy to mainly appressed	Downy to mainly appressed	15×18	20×45	20×50	20×55
Corn meal	Downy-felty to mainly appressed.	Downy-felty to mainly appressed.	Downy-felty to mainly downy appressed.	Downy-felty to mainly downy appressed.	18×25	20×42	20×50	20×56
Prune	Downy-felty to downy	Subfelty to downy	Felty to downy	Felty to downy	20×20	20×55	20×65	20×72
Alfalfa	Subfelty to silky	Silky-felty to appressed	Silky-felty to appressed	Silky-felty to appressed	12×15	20×35	20×60	20×60
Parsnip	Velvety-felty	Felty to appressed	Felty to downy	Felty	15×22	20×45	20×62	20×72
Potato	Downy to appressed	Downy to appressed	Downy to appressed	Downy to felty	15×20	20×30	20×32	20×33

TABLE IX.—*Cultural data for Fomes rimosus from Siderocarpos flexicaulis (FP 21741), Texas*

[Subculture 2 from mycelium on malt agar; initial culture from tissue. Series inoculated on May 31, 1917]

Medium (agar).	Color of aerial mycelium.				Color of submerged mycelium.			
	10 days.	20 days.	30 days.	40 days.	10 days.	20 days.	30 days.	40 days.
Carrot	Antimony yellow to light buff.	Clay color to warm buff.	Clay color	Amber brown	Colorless	Colorless	Colorless	Colorless.
Malt	Clay color to warm buff.	Amber brown to white.	Amber brown to buckthorn brown.	do	Old mycelium buckthorn brown.	Amber brown to colorless.	Amber brown to colorless.	Amber brown to colorless.
Beet	Sodden	Light buff to sodden.	Light buff to mainly sodden.	Light buff to mainly sodden.	Colorless	Colorless 20-22 mm. beyond aerial mycelium.	Colorless 25-30 mm. beyond aerial mycelium.	Colorless 20-30 mm. beyond aerial mycelium.
Celery	Clay color to light buff.	Clay color to colorless.	Buckthorn brown to colorless.	Buckthorn brown to colorless.	do	do	Colorless 15-20 mm. beyond aerial mycelium.	Colorless 20-25 mm. beyond aerial mycelium.
Bean	Light buff	Light buff to mainly sodden.	Light buff to mainly sodden.	Light buff to mainly sodden.	do	do	do	Colorless 15-20 mm. beyond aerial mycelium.
Corn meal	Sayal brown to colorless.	Buckthorn brown to colorless.	Buckthorn brown to colorless.	Buckthorn brown to colorless.	do	Clay color	Clay color to colorless.	Clay color to colorless.
Prune	Clay color to colorless.	do	do	Amber brown	do	Cinnamon brown to colorless.	Cinnamon brown to colorless.	Cinnamon brown to colorless.
Alfalfa	Clay color to light buff.	Buckthorn brown to sodden.	Buckthorn brown to sodden.	Buckthorn brown tosodden.	do	Colorless 10-12 mm. beyond aerial mycelium.	Colorless 6-8 mm. beyond aerial mycelium.	Colorless 20 mm. beyond aerial mycelium.
Parsnip	Warm buff to colorless.	Argus brown to to light buff.	Argus brown	Argus brown	do	Mars brown to colorless.	Mars brown to colorless.	Mars brown to colorless.
Potato	do	Warm buff to colorless.	Warm buff to colorless.	Warm buff to colorless.	do	Colorless	Colorless	Colorless.

Medium (agar).	Texture of aerial mycelium.				Size of area.			
	10 days.	20 days.	30 days.	40 days.	10 days.	20 days.	30 days.	40 days.
					Mm.	*Mm.*	*Mm.*	*Mm.*
Carrot	Subfelty to silky	Felty	Felty	Felty	20×32	20×62	20×72	20×72
Malt	Downy-felty to appressed	Felty to downy	Felty to downy	do	20×25	20×60	20×75	20×75
Beet	Appressed	Downy to mainly appressed	Downy mainly appressed	Downy mainly appressed	15×15	20×32	20×55	20×60
Celery	Subfelty	Felty to appressed	Felty to appressed	Felty to appressed	16×16	20×40	20×55	20×60
Bean	Downy	Downy to appressed	Downy to mainly appressed	Downy to mainly appressed	15×15	20×40	20×55	20×60
Corn meal	Downy-felty to appressed	Downy-felty to appressed	Downy-felty to appressed	Downy-felty to downy	18×20	20×45	20×60	20×65
Prune	do	Subfelty to appressed	Felty to appressed	Felty to downy	20×25	20×50	20×70	20×75
Alfalfa	Subfelty to silky	Felty to appressed	do	Felty to appressed	10×12	20×30	20×50	20×65
Parsnip	Downy to appressed	Subfelty to downy	Felty to downy	Felty	10×12	20×45	20×65	20×75
Potato	do	Downy to appressed	Downy to appressed	Downy to appressed	20×15	20×25	20×26	20×26

TABLE X.—*Cultural data for Fomes rimosus from Prosopis juliflora (FP 21687), Texas*

[Subculture 2 from mycelium on beet agar; initial culture from tissue. Series inoculated May 31, 1917]

Medium (agar).	Color of aerial mycelium.				Color of submerged mycelium.			
	10 days.	20 days.	30 days.	40 days.	10 days.	20 days.	30 days.	40 days.
Carrot	Antimony yellow to light buff.	Clay color to warm buff.	Clay color to warm buff.	Amber brown to antimony yellow.	Colorless	Colorless	Colorless	Colorless.
Malt	Clay color to warm buff.	Amber brown to white.	Amber brown to buckthorn brown.	Amber brown to light buff.	Buckthorn brown.	Amber brown to colorless.	Amber brown to colorless.	Amber brown to colorless.
Beet	Sodden	Light buff to sodden.	Light buff to mainly sodden.	Light buff to mainly sodden.	Colorless	Colorless 10–15 mm. beyond aerial mycelium.	Colorless 20–25 mm. beyond aerial mycelium.	Colorless 20–25 mm. beyond aerial mycelium.
Celery	Light buff to colorless.	Light buff to colorless.	Light buff to colorless.	Light buff to colorless.	do	Colorless 15–20 mm. beyond aerial mycelium.	Colorless 20–25 mm. beyond aerial mycelium.	Colorless 25–30 mm. beyond aerial mycelium.
Bean	Light buff	Light buff to sodden.	Light buff to sodden.	Light buff to sodden.	do	Colorless 10–15 mm. beyond aerial mycelium.	Colorless 15–20 mm. beyond aerial mycelium.	Colorless 10–15 mm. beyond aerial mycelium.
Corn meal	Clay color to colorless.	Clay color to colorless.	Clay color to colorless.	Clay color to colorless.	do	Clay color to mainly colorless.	Clay color to mainly colorless.	Clay color to mainly colorless.
Prune	Light buff to colorless.	Light buff to white.	Clay color to white.	Clay color to white.	do	Ochre to colorless.	Ochre to colorless.	Ochre to colorless.
Alfalfa	Clay color to light buff.	Buckthorn brown to light buff.	Buckthorn brown to colorless.	Buckthorn brown to colorless.	do	Colorless 10–12 mm. beyond aerial mycelium.	Colorless 6–8 mm. beyond aerial mycelium.	Colorless 6–8 mm. beyond aerial mycelium.
Parsnip	Colorless	Clay color to mainly light buff.	Clay color to mainly light buff.	Buckthorn brown to light buff.	do	Colorless	Colorless	Tawny to mainly colorless.
Potato	Warm buff to colorless.	Warm buff to colorless.	Warm buff to colorless.	Warm buff to colorless.	do	do	do	Colorless.

Medium (agar).	Texture of aerial mycelium.				Size of area.			
	10 days.	20 days.	30 days.	40 days.	10 days.	20 days.	30 days.	40 days.
					Mm.	*Mm.*	*Mm.*	*Mm.*
Carrot	Silky-felty to silky	Felty	Felty	Felty	20×35	20×65	20×70	20×70
Malt	Downy-felty to appressed	Felty to downy	Felty to downy	do	20×26	20×50	20×60	20×65
Beet	Appressed	Downy to mainly appressed.	Downy to mainly appressed.	Downy to mainly ap-	8×10	20×32	20×40	20×45
Celery	Downy to appressed	Downy to appressed	Downy to appressed	Downy to appressed	20×30	20×50	20×70	20×70
Bean	Downy	do	do	do	15×20	20×30	20×35	20×42
Corn meal	Downy to appressed	do	do	do	18×20	20×45	20×55	20×57
Prune	do	Subfelty to downy	Subfelty to downy	Subfelty to downy	20×25	20×60	20×70	20×72
Alfalfa	Subfelty to silky	Felty to appressed	Felty to appressed	Felty to appressed	10×18	20×42	20×55	20×56
Parsnip	Appressed	Subfelty to downy	Felty to downy	Felty to downy	10×15	20×50	20×70	20×75
Potato	Downy to appressed	Downy to appressed	Downy to appressed	Downy to appressed	20×20	20×30	20 35	20×36

TABLE XI.—*Cultural data for Fomes robiniae from Robinia neomexicana (FP 21779), New Mexico*

[Subculture 2 from mycelium on parsnip agar; initial culture from infected wood. Series inoculated May 31, 1917]

Medium (agar).	Color of aerial mycelium.				Color of submerged mycelium.			
	10 days.	20 days.	30 days.	40 days.	10 days.	20 days.	30 days.	40 days.
Carrot	Antimony yellow to light buff.	Clay color to warm buff.	Clay color to warm buff.	Clay color to antimony yellow.	Colorless	Colorless	Colorless	Colorless.
Malt	Clay color to light buff.	Amber brown to white.	Amber brown to buckthorn brown.	Amber brown	do	Amber brown	Amber brown to colorless.	Amber brown to colorless.
Beet	Light buff to sodden.	Light buff to mainly sodden.	Light buff to mainly sodden.	Light buff to mainly sodden.	do	Colorless 20–25 mm. beyond aerial mycelium.	Colorless 35–40 mm. beyond aerial mycelium.	Colorless 35–40 mm beyond aerial mucelium.
Celery	Clay color to colorless.	Buckthorn brown to colorless.	Buckthorn brown to mainly colorless.	Buckthorn brown to mainly colorless.	do	do	do	Do.
Bean	Colorless	Light buff to sodden.	Light buff ro sodden.	Light buff to sodden.	do	do	do	Colorless 20–25 mm. beyond aerial mycelium.
Corn meal	Clay color to colorless.	Clay color to colorless.	Clay color to colorless.	Clay color to colorless.	do	Clay color to colorless.	Clay color to colorless.	Clay color to colorless.
Prune	do	Buckthorn brown to colorless.	Buckthorn brown to colorless.	Amber brown to colorless.	do	Cinnamon brown to colorless.	Cinnamon brown to colorless.	Cinnamon brown to colorless.
Alfalfa	Clay color to colorless.	do	Buckthorn brown to light buff.	Buckthorn brown to light buff.	do	Colorless 5–8 mm. beyond aerial mycelium.	Colorless 6–8 mm. beyond aerial mycelium.	Colorless 6–8 mm. beyond aerial mycelium.
Parsnip	Buckthorn brown to warm.	Amber brown to light buff.	Sandford brown to warm buff.	Sandford brown to warm buff.	do	Mars brown to colorless.	Mars brown to colorless.	Mars brown to colorless.
Potato	Warm buff to colorless.	Warm buff to colorless.	Warm buff to colorless.	Warm buff to colorless.	do	Colorless	Colorless	Colorless.

Medium (agar).	Texture of aerial mycelium.				Size of area.			
	10 days.	20 days.	30 days.	40 days.	10 days.	20 days.	30 days.	40 days.
					mm.	*mm.*	*mm.*	*mm.*
Carrot	Felty	Felty	Felty	Felty	20×40	20×70	20×75	20×75
Malt	Subfelty	Felty to downy	do	do	20×35	20×65	20×80	20×80
Beet	Downy-silky to appressed	Downy to mainly appressed.	Downy to mainly appressed.	Downy to mainly appressed.	15×15	20×50	20×60	20×65
Celery	Downy to appressed	Downy to appressed	Subfelty to appressed	Subfelty to appressed	20×28	20×55	20×75	20×75
Bean	Appressed	Appressed	Downy to mainly appressed.	Downy to mainly appressed.	20×25	20×35	20×50	20×52
Corn meal	Downy-felty to appressed	Downy-felty to appressed	Downy-felty to appressed	Downy-felty to appressed	20×32	20×52	20×62	20×63
Prune	do	Subfelty to downy	Subfelty to downy	Subfelty to downy	20×35	20×65	20×75	20×75
Alfalfa	Subfelty to silky	Felty to appressed	Felty to appressed	Felty to appressed	15×18	20×42	20×58	20×58
Parsnip	Velvety-felty	Felty to downy	Felty to downy	Felty to downy	20×28	20×60	20×82	20×82
Potato	Downy to appressed	Downy to appressed	Downy to appressed	Downy to appressed	18×20	20×30	20×35	20×36

TABLE XII.—*Cultural data for Fomes rimosus from Juglans rupestris(?) (FP 21611), Arizona*

[Subculture 3 from mycelium on carrot agar; initial culture from tissue. Series inoculated May 29, 1917]

Medium (agar).	Color of aerial mycelium.				Color of submerged mycelium.			
	10 days.	20 days.	30 days.	40 days.	10 days.	20 days.	30 days.	40 days.
Carrot	Antimony yellow to warm buff.	Clay color to warm buff.	Clay color to warm buff.	Clay color to warm buff.	Colorless	Colorless	Colorless	Colorless.
Malt	Clay color to warm buff.	Buckthorn brown to antimony yellow to warm buff.	Buckthorn brown.	Buckthorn brown.	do	Amber brown	Amber brown	Amber brown.
Beet	Warm buff to sodden.	Warm buff to sodden.	Buckthorn brown to sodden.	Buckthorn brown to sodden.	do	Colorless 10 - 15 mm. beyond aerial mycelium.	Colorless 15 - 20 mm. beyond aerial mycelium.	Colorless 10-12 mm. beyond aerial mycelium.
Celery	Warm buff to colorless.	Warm buff to colorless.	Warm buff to mainly colorless.	Warm buff to mainly colorless.	do	Colorless 20 - 30 mm. beyond aerial mycelium.	Colorless 35 - 40 mm. beyond aerial mycelium.	Colorless 40 - 45 mm. beyond aerial mycelium.
Bean	Light buff	Light buff to mainly sodden.	Light buff to mainly sodden.	Light buff to mainly sodden.	do	Colorless 10 - 15 mm. beyond aerial mycelium.	Colorless 20 - 30 mm. beyond aerial mycelium.	Colorless 20 - 25 mm. beyond aerial mycelium.
Corn meal	Warm buff to colorless.	Warm buff to colorless.	Warm buff to colorless.	Warm buff to colorless.	do	Colorless	Colorless	Colorless.
Prune	Light buff to colorless.	do	Clay color to white	Clay color to white	do	Cinnamon brown to colorless.	Cinnamon brown to colorless.	Cinnamon brown colorless.
Alfalfa	Light buff	Warm buff to colorless.	Warm buff to colorless.	Warm buff to colorless.	do	Colorless 10 - 15 mm. beyond aerial mycelium.	Colorless 20 - 25 mm. beyond aerial mycelium.	Colorless 20 - 25 mm. beyond aerial mycelium.
Parsnip	Antimony yellow to colorless.	Sandford brown to to warm buff.	Sandford brown to to warm buff.	Sandford brown to to warm buff.	do	Mars brown to colorless.	Mars brown to colorless.	Mars brown to colorless.
Potato	Light buff to colorless.	Light buff to colorless.	Light buff to mainly colorless.	Light buff to mainly colorless.	do	Colorless	Colorless	Colorless.

Medium (agar).	Texture of aerial mycelium.				Size of area.			
	10 days.	20 days.	30 days.	40 days.	10 days.	20 days.	30 days.	40 days.
					Mm.	*Mm.*	*Mm.*	*Mm.*
Carrot	Felty to silky	Felty	Felty	Felty	20×35	20×72	20×80	20×80
Malt	Felty to appressed downy	Felty to downy	Felty to downy	Dense felty to downy	20×22	20×60	20×85	20×85
Beet	Silky	Downy to mainly appressed.	Downy to mainly oppressed.	Downy to mainly appressed.	5×10	10×20	20×30	20×31
Celery	Downy to appressed	do	do	do	10×15	20×35	20×50	20×75
Bean	Downy	do	do	do	8×10	20×26	20×40	20×42
Corn meal	Downy to mainly appressed.	Downy to mainly appressed.	do	do	15×18	20×35	20×60	20×70
Prune	Downy to appressed	Downy to appressed	Downy-appressed	Downy-appressed	15×18	20×48	20×70	20×72
Alfalfa	Cottony	Silky-felty to appressed	Silky-felty to appressed	Silky-felty to mainly appressed.	10×15	20×30	20×40	20×60
Parsnip	Felty	Felty to appressed	Felty to appressed	Felty to appressed	20×25	20×55	20×85	20×85
Potato	Downy to appressed	Downy to appressed	Downy to appressed	Downy to appressed	15×12	20×25	20×40	20×45

TABLE XIII.—*Cultural data for Polyporus dryophilus from Quercus gambelii (FP 21598), New Mexico*

[Subculture 2 from mycelium on beet agar; initial culture from infected wood. Series inoculated on June 1, 1917]

Medium (agar).	Color of aerial mycelium.				Color of submerged mycelium.			
	10 days.	20 days.	30 days.	40 days.	10 days.	20 days.	30 days.	40 days.
Carrot	Buckthorn brown to light buff.	Buckthorn brown.	Buckthorn brown.	Buckthorn brown.	Colorless	Colorless	Cinnamon brown to colorless.	Cinnamon brown to colorless.
Malt	do	do	do	do	do	Tawny to colorless.	Tawny	Tawny to colorless.
Beet	White	Light buff to white	Light buff to white	Buckthorn brown to white.	do	Colorless	Colorless	Colorless.
Celery	Light buff to white	do	Warm buff to white.	Warm buff to mainly white.	do	do	do	Do.
Bean	White	do	Light buff to white	Light buff to white	do	do	do	Do.
Corn meal	Clay color to mainly light buff.	Clay color to mainly light buff.	Clay color to mainly light buff.	Clay color to mainly light buff.	do	do	do	Do.
Prune	Warm buff to light buff.	Clay color to light buff.	Clay color to light buff.	Buckthorn brown.	do	Tawny to mainly colorless.	Tawny to mainly colorless.	Tawny to mainly colorless.
Alfalfa	White	Light buff to white	Light buff to white	Light buff to white	do	Colorless	Colorless	Colorless.
Parsnip	Buckthorn brown to light buff.	Buckthorn brown.	Buckthorn brown.	Buckthorn brown.	Tawny	Tawny	Tawny	Tawny.
Potato	White	White	White	White	Colorless	Colorless	Colorless	Colorless.

Medium (agar).	Texture of aerial mycelium.				Size of area.			
	10 days.	20 days.	30 days.	40 days.	10 days.	20 days.	30 days.	40 days.
					Mm.	*Mm.*	*Mm.*	*Mm.*
Carrot	Dense woolly	Dense woolly	Dense woolly	Dense woolly	20×52	20×80	20×80	20×80
Malt	do	do	do	do	20×50	20×75	20×75	20×75
Beet	Cottony	Cottony	Cottony-woolly	Cottony-woolly	18×25	20×45	20×55	20×55
Celery	do	Cottony-woolly	do	do	20×32	20×52	20×56	20×57
Bean	do	Cottony to downy	Cottony-felty	Cottony-felty	20×28	20×50	20×65	20×70
Corn meal	do	Cottony-woolly	Cottony-woolly	Woolly	20×46	20×75	20×75	20×80
Prune	Woolly	Woolly	Woolly	do	20×40	20×80	20×80	20×80
Alfalfa	Woolly to silky	Woolly-silky	Woolly-silky	Woolly-silky	20×28	20×50	20×60	20×61
Parsnip	Woolly, dense	Woolly, dense	Woolly, dense	Woolly, dense	20×50	20×80	20×80	20×80
Potato	Cottony to silky	Cottony to downy	Cottony to downy	Cottony to downy	20×25	20×27	20×28	20×29

TABLE XIV.—*Cultural data for Polyporus texanus from Prosopis juliflora (FP 21201), Texas*

[Subculture 1 from mycelium on malt agar; initial culture from tissue. Series inoculated on May 29, 1917]

Medium (agar).	Color of aerial mycelium.				Color of submerged mycelium.			
	10 days.	20 days.	30 days.	40 days.	10 days.	20 days.	30 days.	40 days.
Carrot	Clay color to light buff.	Clay color to light buff.	Clay color to light buff.	Clay color to light buff.	Colorless	Colorless	Colorless	Colorless.
Malt	do	Buckthorn brown to light buff.	Buckthorn brown to light buff.	Buckthorn brown to light buff.	do	do	do	Do.
Beet	White	White	Light buff to white	Light buff to sodden.	do	do	do	Do.
Celery	Light buff to white	Clay color to white	Clay color to white	Clay color to white	do	do	do	Do.
Bean	Light buff	Light buff	Light buff	Light buff	do	do	do	Do.
Corn meal	Ochraceous buff to light buff.	Clay color to light buff.	Clay color to light buff.	Clay color to white	do	do	do	Do.
Prune	Clay color to light buff.	Clay color to white	Clay color to white	Clay color	do	do	do	Do.
Alfalfa	White	Warm buff to white.	Warm buff to white.	Warm buff to white.	do	do	do	Do.
Parsnip	Clay color to light buff.	Clay color to white	Clay color to white	Buckthorn brown to clay color.	do	do	do	Do.
Potato	Warm buff to white.	Clay color to light buff.	Clay color to light buff.	Clay color to mainly light buff.	do	do	do	Do.

Medium (agar).	Texture of aerial mycelium.				Size of area.			
	10 days.	20 days.	30 days.	40 days.	10 days.	20 days.	30 days.	40 days.
					Mm.	*Mm.*	*Mm.*	*Mm.*
Carrot	Cottony, coarse plumose	Cottony to felty	Cottony to felty	Cottony to felty	20×30	20×50	20×60	20×67
Malt	do	Cottony to cottony-felty	Cottony-felty	Felty strong plumose tufts.	20×28	20×50	20×65	20×75
Beet	Cottony	Cottony	do	Felty to appressed	8×12	15×18	20×22	20×25
Celery	Cottony, plumose	Cottony-felty	do	Felty to downy, plumose tufts.	20×20	20×37	20×45	20×50
Bean	Cottony	Cottony, plumose	Cottony, plumose	Cottony, plumose	8×8 [a]	8×8 [a]	8×8 [a]	8×8 [b]
Corn meal	Cottony, plumose	Cottony	Cottony to felty	Felty to downy, plumose tufts.	15×20	20×48	20×65	20×70
Prune	do	Cottony-felty to downy	Cottony-felty to downy	Felty to downy strong plumose tufts.	20×30	20×50	20×65	20×68
Alfalfa	do	Cottony	Cottony to felty	Felty to downy, plumose tufts.	15×20	20×35	20×45	20×47
Parsnip	do	Cottony to felty-cottony	do	Felty, plumose tufts	20×28	20×54	20×65	20×75
Potato	do	do	do	Felty to downy	20×25	20×40	20×60	20×70

[a] Mainly on inoculum.

[b] Practically no growth.

Tables VII to XII show the cultural characters on the 10 agars for Fomes *rimosus* (two strains) from *Acacia roemeriana* (catclaw), *F. rimosus* from *Siderocarpos flexicaulis* (Texas ebony), *F. rimosus* from *Prosopis juliflora* (mesquite), *F. robiniae* from *Robinia neomexicana* and a species of Fomes from *Juglans rupestris* (?). A study of these six tables fails to show any marked and constant differences in cultural characters for any of the 10 agars for the six strains given, although four of the six strains represent *Fomes rimosus*, one *F. robiniae* and one a Fomes on *Juglans rupestris* (?) which the writers have been referring to *F. everhartii*. Some of the cultures on carrot, malt, and parsnip show slight color differences, but none great enough to be of any specific value. On parsnip and malt, the submerged mycelium for all of these strains is colored, while carrot has colored to colorless mycelium. The dominant characteristics of *Fomes rimosus* is seen on beet, celery, bean, and alfalfa agars. On each of these agars there is but little really aerial mycelium which is downy to mainly appressed, while the submerged mycelium extends far beyond the aerial mycelium, giving a peculiar and very characteristic glassy appearance to the surface of the agar. This peculiarity of growth of the submerged mycelium on these four agars is seen in all of the six strains and indicates that they are one and the same species—viz, that *Fomes robiniae* is only a form of *F. rimosus*, as many scientists have always believed, and that the specimen of Fomes sp. supposed to have been collected on *Juglans rupestris* also belongs to this species. These six examples show how valuable the cultural characters can become in determining the specific position of closely related or identical species. Only one viable sporophore of the Fomes supposed to have been collected on *J. rupestris* was available for culture work, and since this specimen may have been wrongly labeled as to host, the writers do not wish to place the Fomes so common on *J. rupestris* throughout the southwestern United States as belonging positively to *Fomes rimosus* until further cultures with specimens absolutely known to have grown on this host have been made.

The cultural characters of *Fomes everhartii*, *F. arctostaphyli* (*10*), *F. igniarius* from *Populus tremuloides*, and a species of Fomes from Alnus sp., sent to the writers as *F. igniarius*, when compared showed that the Fomes from Alnus is more closely related to *F. arstostaphyli* than to *F. igniarius* from *P. tremuloides*, but that it is apparently neither of these two species; nor did it have any of the cultural characters of *F. everhartii*. In this instance the sporophore from *Alnus* sp. was very similar in all its characters to the sporophore of *F. igniarius* from *P. tremuloides*, yet the cultural characters instantly showed the two were not the same species.

Polyporus farlowii, *P. dryophilus*, and *P. texanus* are three closely related species which differ but little in their general sporophore characters. Tables V, VI, XIII, and XIV show the cultural characters of each

of these three fungi. The cultural differences between *P. texanus* and *P. dryophilus* are much more marked than those between *P. farlowii* and *P. dryophilus*. A study of the tables giving the characters of these three fungi at once shows marked and constant differences between each of the three species. It will be seen that general color resemblances are not as distinctive in differentiating specific characters as are certain other factors. Many of these species have a buckthorn brown color on several of the agars, which indicates their general relationship while specific characters must be sought in the differences of growth on certain agars.

The difference in the fruiting of related species on the various agars is also of much value in differentiating species. For instance, *P. farlowii* fruits vigorously on several of the ten culture media producing perfect and typical pores, *P. dryophilus* very rarely fruits on the culture media here given unless the inoculum is fresh sporophore tissue, while *P. texanus* has so far fruited on only two of them.

IDENTIFICATION OF UNKNOWN ROTS BY CULTURAL CHARACTERS

The cultural character method here given can be used to determine what fungus produces a given rot. In a large majority of cases rots, both heart and saprophytic, will be found without any sporophores being present to indicate what fungus produced the rot. If careful inoculations are made from such infected wood, it is a comparatively easy matter, as a rule, to obtain pure cultures of the causative organisms and later grow them again on the 10 media given, thus determining their cultural characters and from them the fungi producing the rots.

The number of species of fungi producing heartrots in living trees is not very great. A few of them produce rots which can usually be identified by the character of the rot alone, such as *Trametes pini*, but the great majority of them can not be certainly determined by the rot. For instance, the rot produced in conifers by *Polyporus schweinitzii*, *P. sulphureus*, and *Fomes laricis* are so similar that no one can be certain by examining the rot alone which of these three fungi was the cause of the rot in question.

The rots of structural timbers are more numerous than the true heartrots and the causative organism producing each rot is more difficult to determine from the rot alone, since the majority of the structural-timber rots are very similar in general appearance. Most of these rots are of the carbonizing type, where a brownish, brittle rot is produced which on dessication breaks up into little cubical blocks of varying sizes. Pure cultures from these structural timber rots will differentiate them as to their causative organisms at once. The value of such means of determining the causative organism from the rot in the absence of any sporophore is very evident to anyone who has had to deal with such rots.

EXAMPLES OF IDENTIFICATION OF UNKNOWN ROTS BY CULTURAL CHARACTERS

The following examples illustrate how the cultural characters here described may be used to determine the causative organism of unknown rots:

(*a*) There are two common heartrots found in conifers in the western United States. One is the redrot caused by *Trametes pini* and the other is what the senior author has previously called western redrot (*9*). In their early stages of growth these two rots resemble each other very much. Pure cultures from each of them differentiate the two immediately, since the pure cultures of the fungus which causes western redrot, called previously *Polyporus ellisianus*, produces entirely white cultures on all of the 10 media, while the cultures of *Trametes pini* are varying shades of brown.

(*b*) On a recent field trip in eastern Texas, when the senior writer was studying the rots of bridge timbers and railroad ties, he often found a certain rot in driven bridge piling made of creosoted longleaf pine (*Pinus palustris*). At that time in the year there were no fruiting bodies present on the rotting piling and the question at once arose as to what fungus was the cause of this serious rot. From the peculiar odor of the freshly opened wood as well as the character of the rot it was believed that it was caused by *Lentinus lepideus*, but nothing could be determined definitely from an examination of the rot alone. Specimens of the rotted piling were forwarded to the laboratory and cultures made from them. Pure cultures were obtained showing all of the characters of *L. lepideus* when grown on the 10 cultural media. Later many of these cultures developed the typical sporophores of *L. lepideus*. Cultures of this fungus can usually be recognized by the presence of abortive sporophores which develop on the surface of the agar and also by the presence of large, thick-walled, obovate to subglobose spores in the submerged mycelium.

(*c*) While studying the rots of cypress (*Taxodium distichum*) ties in wet locations in eastern Texas two unknown rots were found in this wood. Pieces of the rotting ties were sent to the laboratory and pure cultures of two different fungi were obtained. One set of these cultures showed all the cultural characters of *Lentinus lepideus*, while the other series produced a sporophore of an unknown species of Poria.

(*d*) A rot in the heartwood of *Quercus gambelii*, collected in New Mexico, was determined in the field as caused by *Polyporus dryophilus*. Cultures of the diseased wood gave all of the cultural characters of *Fomes everhartii* and none of those of *P. dryophilus*.

(*e*) A specimen of rot in *Pinus ponderosa*, which was supposed to be caused by *Polyporus sulphureus*, was received from Oregon. Cultures from the wood showed that the rot was unquestionably caused by

Lentinus lepideus. Since the rot was sent in as a specimen of heartrot found in the western yellow pine in Oregon, the writers are wondering whether *L. lepideus* produces a real heartrot of living pine in that State or whether this rot came from a dead area on a living or from a dead tree which had been attacked by this saprophytic fungus.

Many other instances could be cited of the determination of the causative organism of a rot by use of the cultural methods here outlined. Usually it is not even necessary that the sporophore stage should be developed, since the vegative cultural characters on the 10 special media will usually determine the identity of the fungus. The practical importance of such a method of determination is of great value and is easily recognized by anyone who has worked for any length of time with organisms of this character. In fact, one of the worst stumbling blocks to a successful study of the various rots of wood, both saprophytic and heart rots, has been the lack of methods by which the organisms producing these rots could be grown and identified in pure cultures on artificial media.

SPOROPHORE PRODUCTION

The fact has long been known that the production of sporophores in nature in many of the Hymenomycetes was more or less dependent upon light. This fact has also been demonstrated for a few species mainly by gross cultures on dung decoctions, pieces of wood, bread, etc. Buller (*4*) has shown among other things how the light influences sporophore production for a few species, mainly Agaricaceae, but none of his experiments were made with pure cultures on artificial media. It has been taken for granted that light was essential to the formation of sporophores of the wood-rotting fungi, including the Polyporaceae, but such had never been proved with pure cultures under control conditions on artificial media with a sufficiently large number of species to determine the actual influence of light as well as other factors on sporophore production.

The studies here made indicate that there are many Polyporaceae which fruit in diffused light of varying degrees of intensity and others apparently require the direct rays of the sun to produce perfect spore-bearing sporophores, while some can form sporophores in absolute darkness. Since this is only a preliminary report, no attempt is made to determine a large number of factors which should be ascertained in a complete study of this phase of fungus life. For instance, there must be a minimum, optimum, and maximum condition as to light, heat, and moisture under which a given fungus will produce sporophores.

It will be seen by consulting Table XVI that only three species, *Polyporus farlowii*, *Trametes serialis*, and *P. cinnabarinus*, were able to develop sporophores in absolute darkness. Only the first two of these fungi produced both sporophores and spores. *P. cinnabarinus* produced fairly typical pores, but no spores were found.

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METHODS OF EXPERIMENTATION

In the earlier sporophore study the tubes containing the cultures were kept both horizontally and vertically. If the species under investigation produced sporophores at all, they were able to produce them in either position. However, the practice was soon abandoned of placing the tubes in a vertical position, since it was found very difficult to obtain uniformity in the proper lighting of the cultures and to get spore prints from the sporophores produced in such a position.

The general method followed in the sporophore studies was to take a series of tubes on different agars and place them in the same general position that was described in the study of the cultural characters. It was found very important early in the study that the slant side of the tube should be kept uppermost and that the relative position of the tube in reference to gravity and sunlight should be the same throughout the experiment. The tubes were so placed that the cotton plugs were away from the sunlight and the bottom of the tubes faced the light. As soon as there was any indication of a hymenium forming, the tubes were placed with the slanting surface downward. After this was done, the sporophores usually continued to develop normally, and in due season spores would be formed and discharged against the side of the tube opposite the hymenium.

The first sets of fungi which were kept in darkness were placed in pasteboard boxes in a horizontal position and these boxes inclosed in other pasteboard boxes. It was found, however, that some diffused light reached the tubes in spite of the double-box arrangement. Inside one of these boxes the recording tube of the soil thermograph was placed, while the registering portion of the instrument was kept on a shelf outside the boxes. These boxes were kept on the shelf beside the other boxes containing the tubes which were exposed to the direct rays of the sun. This was done in order to obtain as near as possible the same environment for the tubes kept in the sunlight and those kept in the darkness, except for the single factor of light.

In a later series of experiments, the ones which are recorded in detail in this paper, the cultures were kept in pasteboard boxes in a horizontal position and these boxes were placed in a photographic dark room from which all light was excluded by means of sheets of cardboard being placed over the ruby lights. By this arrangement absolute darkness was obtained for the cultures. The soil thermograph was arranged in the same manner in the dark room as when on the outside. Table XV shows the highest maximum and minimum, the lowest maximum and minimum, the highest and the lowest mean, and the average of the daily maximum, minimum, and mean temperatures for each month as recorded by the two thermographs.

TABLE XV.—*Temperature records (°F.) for cultures grown in daylight and in darkness*

Date and degree recorded.	In light.			In dark.		
	Maximum.	Minimum.	Mean.	Maximum.	Minimum.	Mean.
December 18–31, 1916:						
Highest	80	66	73	75	65	69
Lowest	72	55	65	66	57	64
Average	76	61	68	71	61	66
January, 1917:						
Highest	89	70	77	78	73	74
Lowest	65	52	58	63	58	60
Average	79	60	69	71	68	69
February, 1917:						
Highest	97	67	81	86	72	78
Lowest	74	56	66	68	59	65
Average	87	60	73	77	65	71
March, 1917:						
Highest	96	72	80	88	80	83
Lowest	71	50	62	73	62	68
Average	86.5	59	72.7	79	68	73
April, 1917:						
Highest	96	66	77	86	80	80
Lowest	69	46	65	68	65	67
Average	82	59	70.5	78.7	70.8	74.7
May, 1917:						
Highest	100	67	83	77	74	75
Lowest	61	49	56	60	57	59
Average	80	60	70	71	68	69.5
June, 1917:						
Highest	110	78	93	92	88	90
Lowest	82	50	68	70	69	70
Average	96	70	83	84	80	82

EXPLANATION OF TERMS USED IN SPOROPHORE TABLE XVI.

In Table XVI most of the headings are self-explanatory; however, there are a few that need special explanation. The heading for the sixth column is "Inoculum and date of inoculation." Under this heading the character of the inoculum used when sphorophores were produced is given, as well as the date or dates when each tube which produced sphorophores was inoculated. "Wood" means the inoculum used was infected wood; "tissue" means pieces of sphorophore tissue, while "malt," "potato," etc., signifies that mycelium was used as an inoculum and that it was taken from a culture on malt agar, potato agar, etc. "Inoculum as used means the piece of wood, tissue, etc., used to inoculate the media. "Development period of sporophores" means the number of days from the time the inoculation was made to the first evidence of the formation of a hymenium.

Table XVI shows that the authors have obtained under the cultural methods described 629 sporophores in the light and 11 in darkness, 640 in all, representing 4 families (Agaricaceae, Polyporaceae, Thelephoraceae, and Tremellaceae), 16 genera (Coprinus, Daedalea, Exidea, Fomes, Ganoderma, Irpex, Lentinus, Lenzites, Merulius, Panus (?) Pleurotus, Polyporus, Polystictus, Poria, Stereum, and Trametes), 42 species, and 97 strains from 65 host species collected in 11 States. If the genera given in North American Flora are used, there would be 24 genera, since the following 8 genera would be added: Coriollelus, Elfvingia, Inonotus, Laetiporus, Pycnoporus, Pyropolyporus, Spongipellis, and Tyromyces.

TABLE XVI.—*Data relating to sporophore production on artificial media*

Forest Pathology No.	Name of fungus.	State where collected.	Host.	Source of initial inoculum.	Inoculum and date of inoculation.	
					In light.	In dark.
21831	*Coprinus atramentarius*	Montana	*Populus trichocarpa*	Mycelium	Tissue, May 2, 1917	
21562	*Daedalea juniperina*	New Mexico	*Juniperus pachyphloea*	Wood	Wood, May 12, 1917	
21592	*Daedalea juniperina*	Arizona	*Juniperus utahensis*	do	Prune, Mar. 16, 1917	Carrot, Apr. 7, 1917.
21874	*Exidia recisa*	New Mexico	*Quercus gambelii*	do	Wood, May 24, 1917	
21830	*Fomes applanatus*	Colorado	*Populus trichocarpa*	Tissue	Tissue, May 29, 1917	
21602	*Fomes arctostaphyli*	Arizona	*Arctostaphylos pungens*	Wood	Wood, Oct. 24, 1916	Malt, Dec. 18, 1916.
21776	*Fomes laricis*	do	*Pinus ponderosa*	do	Prune, Mar. 14, 1917	
21756	*Fomes meliae* (?)	Texas	*Melia azedarach*	do	Wood, Jan. 11, 1917	Potato, Apr. 7, 1917.
21838	*Fomes pinicola*	Idaho	*Larix occidentalis*	Tissue	Parsnip, June 7, 1917	
21488	do	New Mexico	*Pseudotsuga taxifolia*	do	Malt, Dec. 20, 1916	Malt, Dec. 20, 1916.
21817	*Fomes rimosus*	Texas	*Acacia roemeriana*	Wood	Wood, Apr. 10, 1917	
21087	do	do	*Prosopis juliflora*	Tissue	Corn meal, June 2, 1917	
21741	do	do	*Siderocarpos flexicaulis*	do	Prune, June 1, 1917	
21611	do	Arizona	*Juglans rupestris* (?)	do	Tissue, Apr. 7, 1917	
21884	*Fomes robiniae*	New Mexico	*Robinia neomexicana*	do	Tissue and malt, May 24 and June 9, 1917.	
19114	*Fomes roseus*	do	*Populus tremuloides*	do	Tissue, May 26, 1917	
19534	do	do	*Picea parryana*	Wood	Wood, May 26, 1917	
21290	do	do	*Pinus edulis*	Tissue	Tissue, May 24, 1917	
21423	do	do	do	do	do	
21277	do	do	*Pseudotsuga taxifolia*	do	do	
21376	do	do	do	do	Tissue, May 26, 1917	
21835	do	Oregon	do	do	Tissue, May 2, 1917	
21880	*Fomes scutellatus*	New Mexico	*Quercus gambelii*	do	Tissue, May 24, 1917	
21880b	do	do	do	Wood	Wood, May 24, 1917	
21807	*Fomes texanus*	do	*Juniperus monosperma*	Tissue	Tissue Feb. 25, 1917; alfalfa, Mar. 12, 1917.	Prune, Apr. 7, 1917.
21653	do	do	do	do	Corn meal, Dec. 6, 1916, June 1, 1917.	Malt, Dec. 6, 1916, Apr. 7, 1917.
21806	do	do	*Juniperus scopulorum*	do	Tissue, Feb. 25, 1917	Prune, Apr. 7, 1917.
21760	do	do	do	do	Corn meal, Mar. 12, 1917	Corn meal, Apr. 7, 1917.
21729	*Ganoderma sessile*	Texas	*Quercus nigra*	do	Tissue, Jan. 27, 1917	Tissue, Jan. 27, 1917.
21775	*Irpex lacteus*	New Mexico	*Quercus arizonica*	Wood	Corn meal, Mar. 23, 1917	
21706	*Lentinus lepideus*	Texas	*Pinus palustris*	do	Wood, Nov. 25, 1916; prune, Mar. 12, 1917.	Wood, Nov. 25, 1916; corn meal, Apr. 7, 1917.
21587	*Lenzites saepiaria*	Arizona	*Pinus ponderosa*	do	Corn meal, Dec. 20, 1916	Corn meal, Dec. 20, 1916.
21878	do	do	do	do	Wood, May 24, 1917	
21794	*Merulius ambiguus*	New Mexico	*Pinus edulis*	do	Prune, Apr. 16, 1917; wood, Feb. 12, 1917.	Prune, Apr. 7, 1917.
21768	*Panus* sp. (?)	do	*Abies concolor*	do	Malt, Mar. 14, 1917	Alfalfa, Apr. 7, 1917.
21871	*Pleurotus ostreatus*	Colorado	*Populus* sp.	Tissue	Tissue, May 17, 1917	

TABLE XVI.—*Data relating to sporophore production on artificial media*—Continued

Forest Pathology No.	Name of fungus.	State where collected.	Host.	Source of initial inoculum.	Inoculum and date of inoculation.	
					In light.	In dark.
21747	*Pleurotus ostreatus*	New Mexico	*Populus wislizeni*	Tissue	Tissue, Dec. 28, 1916; corn meal, Jan. 6, 1917; spores, Jan. 17, 1917.	Bean, Jan. 17, 1917; spores, Apr. 7, 1917.
21586	*Polyporus* sp	Arizona	*Populus tremuloides*	do	Malt, Dec. 20, 1916, Apr. 17, 1917.	Malt, Dec. 20, 1916, Apr. 7, 1917.
21917	do	Texas	*Prunus persica*	Wood	Wood, June 8, 1917	
21919	do	New Mexico	*Acer negundo*	do	Wood, June 26, 1917	
21785	*Polyporus albidus*	Idaho	*Pinus ponderosa*	Tissue	Tissue, Feb. 2, 1917; potato, Apr. 18, 1917.	
21800	*Polyporus anceps*	do	do	do	Tissue, Jan. 29, 1917; potato, Apr. 17, 1917.	
21801	do	do	*Thuja plicata*	do	Tissue, Jan. 29, 1917; prune, Apr. 17, 1917.	
19867	*Polyporus cinnabarinus*	Arkansas	*Quercus alba*	Wood	Tissue, Mar. 9, 1917; malt, Feb. 14 and June 2, 1917.	Potato, Apr. 7, 1917.
21514	do	New Mexico	*Quercus gambelii*	do	Tissue, Feb. 14, 1917; potato, Mar. 9 and June 2, 1917.	Bean, Apr. 7, 1917.
21856a	do	do	do	do	Wood, May 12, 1917	
19001	do	Florida	*Quercus nigra*	do	Tissue, Feb. 14, 1917; malt, Mar. 9 and June 2, 1917.	Carrot, Apr. 17, 1917.
19793	do	Arkansas	*Quercus velutina*	do	Tissue, Feb. 7, 1917; prune, Mar. 9 and June 2, 1917.	Bean, Apr. 7, 1917.
21759	do	New Mexico	*Pinus edulis*	do	Tissue, Jan. 21, 1917; potato, Mar. 9 and June 2, 1917.	Potato, Apr. 7, 1917.
21484	*Polyporus dryophilus*	do	*Quercus gambelii*	do	Tissue, Jan. 29, 1917; prune, Mar. 10, 1917.	Prune, Apr. 7, 1917.
21789	do	Arizona	do	do	Corn meal, Mar. 9, 1917	Corn meal, Apr. 7, 1917.
21936	do	do	do	Tissue	Tissue, July 25, 1917	
21964	do	do	do	do	Tissue, Aug. 18, 1917	
21938	do	New Mexico	*Quercus arizonica*	do	Tissue, July 21, 1917	
21825	*Polyporus ellisianus* (?)	Idaho (?)	*Abies grandis*	do	Tissue, Feb. 2, 1917; potato, Apr. 17, 1917.	
21272	do	New Mexico	*Pinus edulis*	do	Tissue, Feb. 13, 1917	
21795	do	do	do	do	Corn meal, Feb. 13, 1917	
21352	do	Arizona	*Pinus ponderosa*	do	Tissue, Feb. 13, 1917; malt, Apr. 18, 1917.	Malt, Apr. 18, 1917.
21305	do	do	do	Wood	Corn meal, Jan. 9, 1917	Corn meal, Jan. 9, 1917.
21304	do	do	do	do	Corn meal, Jan. 9, 1917	Corn meal, Jan. 9, 1917.
19596	do	New Mexico	do	Tissue	Tissue, Feb. 13, 1917; prune, Apr. 17, 1917.	
21787	do	Arizona	do	Wood	Corn meal, Apr. 18, 1917	

21752	do	New Mexico	do	do	Wood, Jan. 20, 1917; corn meal, Apr. 18, 1917.	
21750	do	do	do	do	do	
21623	do	Arizona	do	Tissue	Tissue, Feb. 12, 1917; prune Apr. 17, 1917.	
21578	do	do	do	Wood	Wood, Oct. 14, 1916; malt, Dec. 20, 1916, Apr. 18, 1917.	Corn meal, Dec. 20, 1916.
21910	do	Montana	do	Tissue	Tissue, May 18, 1917	
21591	*Polyporus farlowii*	New Mexico	*Acer negundo*	do	Tissue, Mar. 9, 1917; parsnip, Apr. 7, 1917.	Parsnip, Apr. 7, 1917.
21402	do	do	*Populus italica*	do	Tissue, Aug. 24, 1916; potato, Sept. 15, 1916, Apr. 7, 1917.	Potato, Apr. 7, 1917.
12986	*Polyporus obtusas*	North Carolina	*Quercus velutina*	Wood	Wood, May 24, 1917	
21679	*Polyporus sulphureus*	Texas	*Quercus virginiana*	Tissue	Chlamydospores from malt, Dec. 20, 1916, Mar. 15, 1917.	Dec. 20, 1916.
21201	*Polyporus texanus*	do	*Prosopis juliflora*	do	Prune, May 29, 1917	
21805	do	do	do	Wood	Celery, June 1, 1917	
21891	*Polystictus hirsutus*	New Mexico	*Prunus melanocarpa*	do	Wood, May 24, 1917	
21886	do	do	*Quercus gambelii*	do	do	
21906	do	do	do	do	do	
21893	do	do	do	Tissue	Tissue, May 24, 1917	
21908	do	do	*Acer negundo*	Wood	Wood, May 24, 1917	
21901	*Polystictus versicolor*	do	*Quercus gambelii*	do	Wood, May 24, 1917	
21872	do	do	do	Tissue	Tissue, May 24, 1917	
21468	*Poria* sp	do	*Juniperus pachyphloea*	do	Tissue, May 12, 1917	
21580	do	Arizona	*Pinus ponderosa*	do	Tissue, Oct. 4, 1916; malt, Dec. 20, 1916.	Malt, Dec. 20, 1916; Apr. 7, 1917.
29873	do	New Mexico	do	Wood	Wood, May 24, 1917	
21707	do	Texas	*Taxodium distichum*	do	Wood, Nov. 25, 1916	Wood, Nov. 25, 1916; Malt, Apr. 16, 1917.
21397	*Stereum umbrinum*	Arkansas	*Quercus alba*	do	Corn meal, Apr. 16, 1917	Corn meal, Apr. 16, 1917.
19905	do	do	do	do	Corn meal, Mar. 16, 1917	Potato, Apr. 7, 1917.
19895	do	do	do	do	do	Bean, Apr. 7, 1917.
19783	*Stereum versiforme*	do	do	do	do	Corn meal, Apr. 7, 1917.
21870	*Trametes peckii*	Colorado	*Populus* sp	Tissue	Tissue, May 12, 1917; Carrot, June 7, 1917.	
21948	do	New Mexico	*Populus angustifolia*	Spores	Spores, Aug. 9, 1917	
21948	do	do	do	Tissue	Tissue, Aug, 17, 1917	
21792	do	do	*Populus wislizeni*	do	Tissue, Feb. 12, 1917	
21791	do	do	do	Wood	Spores, Mar. 12, 1917; Malt, June 7, 1917.	Celery, Apr. 7, 1917.
21761	*Trametes serialis*	do	*Juniperus monosperma*	Tissue	Tissue, Jan. 15 and Mar. 13, 1917; prune, June 2 and June 9, 1917.	Parsnip, Apr. 7, 1917.
21868	do	do	*Juniperus pachyphloea*	Wood	Wood, May 12, 1917; Prune, June 7, 1917.	
21861	do	do	do	do	Wood, May 12, 1917; Malt, June 7, 1917.	
21866a	do	do	*Juniperus scopulorum*	do	do	

Forest Pathology No.	Name of fungus.	State where collected.	Host.	Media producing sporophores.		Number of sporophores produced.		Development period for sporophores.		Color of hymenium.
				Light.	Dark.	Light.	Dark.	Light.	Dark.	
								Days.	Days.	
21831	*Coprinus atramentarius*	Montana	*Populus trichocarpa*	Malt, parsnip	......	2	......	25–31	......	
21862	*Daedalea juniperina*	New Mexico	*Juniperus pachyphloea*	Corn meal, malt, prune.	0	6	......	8–17	......	White.
21592	*D. juniperina*	Arizona	*Juniperus utahensis*	Corn meal	0	4	0	20–21	0	Do.
21874	*Exidea recisa*	New Mexico	*Quercus gambelii*	Carrot, corn meal, prune, malt.	......	6	......	15–30	......	Auburn to carob brown.
21830	*Fomes applanatus*	Colorado	*Populus trichocarpa*	Malt, parsnip	......	3	......	30–38	......	White.
21602	*Fomes arctostaphyli*	Arizona	*Arctostaphylos pungens*	Malt	0	1	0	77	0	Buckthorn brown.
21776	*Fomes laricis*	do	*Pinus ponderosa*	Corn meal	......	1	......	85	......	White.
21756	*Fomes meliae* (?)	Texas	*Melia azedarach*	Corn meal, prune	0	2	0	28	0	White to light buff.
21838	*Fomes pinicola*	Idaho	*Larix occidentalis*	Prune	0	1	0	43	0	White.
21488	do	Mew Mexico	*Pseudotsuga taxifolia*	Corn meal	0	2	0	77	0	Do.
21817	*Fomes rimosus*	Texas	*Acacia roemeriana*	Carrot	......	2	......	52	......	Cinnamon brown.
21687	do	do	*Prosopis juliflora*	Malt	0	3	0	18	0	Do.
21741	do	do	*Siderocarpos flexicaulis*	do	0	1	0	18	0	Do.
21611	do	Arizona	*Juglans rupestris* (?)	Prune	0	3	......	15–33	......	Do.
21884	*Fomes robiniae*	New Mexico	*Robinia neomexicana*	Corn meal, prune	......	5	......	30–40	......	Buckthorn brown.
19114	*Fomes roseus*	do	*Populus tremuloides*	Carrot, parsnip	......	3	......	29	......	Pale cinnamon pink.
19534	do	do	*Picea parryana*	Malt, carrot, corn meal, parsnip, prune.	......	9	......	11–37	......	Shell pink to pale cinnamon pink.
21290	do	do	*Pinus edulis*	Carrot	......	3	......	21–40	......	Pale cinnamon pink.
21423	do	do	do	Prune, parsnip, malt, corn meal.	......	7	......	11–40	......	Shell pink to pale cinnamon pink.
21277	do	do	*Pseudotsuga taxifolia*	Corn meal, carrot, parsnip.	......	5	......	15–20	......	Pale pinkish cinnamon.
21376	do	do	do	Corn meal, carrot, prune, parsnip,	......	6	......	7–23	......	Do.
21835	do	Oregon	do	Prune	0	1	0	10	0	Shell pink.
21880	*Fomes scutellatus*	New Mexico	*Quercus gambelii*	Malt, prune, corn meal, parsnip, carrot.	......	7	......	9–15	......	White.
21880b	do	do	do	Carrot	......	2	......	16	......	Do.
21807	*Fomes texanus*	do	*Juniperus monosperma*	Malt, carrot	0	6	0	14–23	0	Buckthorn brown.
21653	do	do	do	Malt	0	8	0	16–22	0	Do.
21806	do	do	*Juniperus scopulorum*	do	0	1	0	18	0	Do.
21760	do	do	do	do	0	2	0	22–23	0	Do.
21729	*Ganoderma sessile*	Texas	*Quercus nigra*	Corn meal	......	1	0	30	......	
21775	*Irpex lacteus*	New Mexico	*Quercus arizonica*	Beet	......	1	......	88	......	Light buff.
21706	*Lentinus lepideus*	Texas	*Pinus palustris*	Corn meal	......	4	......	22–40	......	

21587	*Lenzites saepiaria*	Arizona	*Pinus ponderosa*	Malt, corn meal	....	9	o	20–31	....	Buckthorn brown.
21878	do	do	do	Corn meal, prune, parsnip.	....	3	....	19–32	....	Do.
21794	*Merulius ambiguus*	New Mexico	*Pinus edulis*	Malt, carrot, celery, bean, prune, corn meal, potato.	....	8	o	14–30	o	Pinkish cinnamon to orange cinnamon.
21768	*Panus* sp. (?)	do	*Abies concolor*	Corn meal, malt	o	12	o	57–62	o	White.
21871	*Pleurotus ostreatus*	Colorado	*Populus* sp.	Malt	....	5	....	37	....	....
21747	do	New Mexico	*Populus wislizeni*	Carrot, malt, beet, corn meal, prune, potato.	o	51	o	16–50	o	White.
21580	*Polyporus* sp.	Arizona	*Populus tremuloides*	Malt, beet, prune, parsnip, carrot, corn meal.	o	14	o	15–23	o	Light buff to warm buff.
21917	do	Texas	*Prunus persica*	Corn meal	....	1	....	12	....	White.
21919	do	New Mexico	*Acer negundo*	Carrot, malt, parsnip, corn meal.	....	6	....	25–41	....	Cinnamon brown.
21785	*Polyporus albidus*	Idaho	*Pinus ponderosa*	Celery, carrot, bean, malt, potato, prune.	....	10	....	13	....	White to light buff.
21800	*Polyporus anceps*	do	do	do	....	13	....	5–33	....	Do.
21801	do	do	*Thuja plicata*	Carrot, celery, malt, bean, corn meal, prune, potato, parsnip.	....	15	....	5–16	....	Do.
19867	*Polyporus cinnabarinus*	Arkansas	*Quercus alba*	Malt, carrot, alfalfa.	o	8	o	17–33	o	Grenadine red to flame scarlet.
21514	do	New Mexico	*Quercus gambelii*	Malt, corn meal, potato, prune.	Malt	8	1	16–33	16	Do.
21850a	do	do	do	Carrot, malt, corn meal.	....	5	....	16–28	....	English red to grenadine red.
19001	do	Florida	*Quercus nigra*	Corn meal, malt	o	6	o	12–18	o	Grenadine red to flame scarlet.
19793	do	Arkansas	*Quercus velutina*	Carrot, celery, malt.	o	7	o	11–22	o	Do.
21759	do	New Mexico	*Pinus edulis*	Malt, celery, prune, potato, carrot, parsnip.	o	6	o	16–33	o	Pores orange chrome to flame scarlet.
21484	*Polyporus dryophilus*	do	*Quercus gambelii*	Corn meal, potato.	o	3	o	10–11	o	Buckthorn brown.
21789	do	Arizona	do	Corn meal, prune, malt.	o	4	o	9–15	o	Do.
21936	do	do	do	Carrot, prune, beet	....	5	....	5–20	....	Do.
21964	do	do	do	Prune	....	2	....	7	....	Do.
21938	do	New Mexico	*Quercus arizonica*	Carrot	....	1	....	6	....	Do.
21825	*Polyporus ellisianus* (?)	Idaho	*Abies grandis*	Carrot, malt, bean, corn meal, prune, potato.	....	10	....	8–12	....	White.
21272	do	New Mexico	*Pinus edulis*	Malt, prune	....	5	....	4–5	....	White to light buff.
21795	do	do	do	Prune	....	1	....	41	....	White.

Forest Pathology No.	Name of fungus.	State where collected.	Host.	Media producing sporophores.		Number of sporophores produced.		Development period for sporophores.		Color of hymenium.
				Light.	Dark.	Light.	Dark.	Light.	Dark.	
								Days.	Days.	
21352	*Polyporus ellisianus* (?)	Arizona	*Pinus ponderosa*	Malt, corn meal, prune, celery, potato, carrot.	0	12	0	8-32	0	White to light buff.
21305	do	do	do	Malt, corn meal	0	6	0	32-34	0	Do.
21304	do	do	do	do	0	5	0	24	0	Do.
19596	do	New Mexico	do	Malt, prune, corn meal, potato.	....	11	....	8-32	....	Do.
21787	do	Arizona	do	Celery, corn meal	....	2	....	11	....	White.
21752	do	New Mexico	do	Malt, corn meal, prune.	....	6	....	25-47	....	Do.
21750	do	do	do	Malt, corn meal, parsnip.	....	7	....	22-59	....	White to warm buff.
21623	do	Arizona	do	Malt, prune	....	4	....	4-25	....	White to light buff.
21578	do	do	do	Malt, corn meal, prune, bean.	0	26	0	12-24	0	Do.
21910	do	Montana	do	Corn meal	....	2	....	4	....	White to cream buff.
21591	*Polyporus farlowii*	New Mexico	*Acer negundo*	Malt, carrot, corn meal, prune, corn beet.	Malt, m ear prune.	12	6	13-38	15-20	Buckthorn brown
21402	do	do	*Populus italica*	Malt, corn meal, prune, beet, parsnip.	Corn meal, pune.	29	3	13-28	20-26	Do.
12986	*Polyporus obtusus*	North Carolina	*Quercus velutina*	Prune, carrot, corn meal.	....	4	....	30-36	....	Warm buff.
21679	*Polyporus sulphureus*	Texas	*Quercus virginiana*	Celery, prune	0	4	0	60	0	Pale orange-yellow
21201	*Polyporus texanus*	do	*Proposis juliflora*	Carrot	....	1	....	39	....	Clay color.
21805	do	do	do	Carrot, parsnip	....	3	....	37-68	....	Do.
21891	*Polystictus hirsutus*	New Mexico	*Prunus melanocarpa*	Carrot, prune, parsnip.	....	5	....	9-10	....	Light buff to warm buff.
21886	do	do	*Quercus gambelli*	Malt, carrot, corn meal, prune, parsnip.	....	10	....	9-12	....	Do.
21906	do	do	do	Malt, prune, carrot, corn meal, parsnip.	....	10	....	8-16	....	Do.
21893	do	do	do	do	....	9	....	8-27	....	Do.
21908	do	do	*Acer negundo*	Malt, prune, corn meal, carrot.	0	8	....	11-25	....	Do.
21901	*Polystictus versicolor*	do	*Quercus gambelii*	Parsnip	....	1	....	16	....	White.
21872	do	do	do	Malt	....	1	....	14	0	Do.

21468	*Poria* sp.	do.	*Juniperus pachyphloea*	Carrot, prune.		3		7–10		Do.
21580	do.	Arizona	*Pinus ponderosa*	Malt, corn meal.	0	8	0	20–33	0	Do.
29873	do.	New Mexico	do.	Carrot, malt, prune		4		21–25		Baryta yellow.
21707	do.	Texas	*Taxodium distichum*	Malt	0	1	0	72	0	Cadmium orange.
21397	*Stereum umbrinum*	Arkansas	*Quercus alba*	Corn meal, parsnip, potato, beet, bean, alfalfa.	0	6	0	11–30	0	Purple drab to brownish drab.
19905	do.	do.	do.	Bean	0	1	0	17	0	Purple drab.
19895	do.	do.	do.	Malt, beet	0	2	0	17–19	0	Cinnamon-drab to brownish drab.
19783	*Stereum versiforme*	do.	do.	Corn meal, beet, parsnip.	0	6	0	25–48	0	Cinnamon-drab to ochraceous tawny.
21870	*Trametes peckii*	Colorado	*Populus* sp.	Carrot, prune, parsnip, bean.		5		7–17		Clay color to wood brown.
21948	do.	New Mexico	*Populus angustifolia*	Carrot, malt		12	0	10–18		Wood brown.
21948	do.	do.	do.	Malt, carrot, corn meal, prune, parsnip.		8		2–6		Do.
21792	do.	do.	*Populus wislizeni*	Prune		4		16–27		Do.
21791	do.	do.	do.	Malt, carrot, beet, prune, parsnip.		19	0	11–28	0	Do.
21761	*Trametes serialis*	do.	*Juniperus monosperma*	Prune, carrot, malt, corn meal, celery, potato.	Prune.	18	1	8–16	17	Light buff.
21868	do.	do.	*Juniperus pachyphloea*	Malt, parsnip, prune.		5		7–12		Do.
21861	do.	do.	do.	Parsnip, carrot, malt, celery, corn meal, bean, potato.		11		7–14		Do.
21866a	do.	do.	*Juniperus scopulorum*	Malt, prune, carrot, corn meal, parsnip, beet, bean, celery, potato.		12		7–18		Light buff to warm buff.

Sporophores were produced when various kinds of inocula were used, such as wood, tissue, spores, or the mycelium from other cultures and on a great variety of artificial culture media. The wide range of genera and species covered by this investigation, together with the large number of sporophores produced, clearly proves that the sporophore production here reported is not an accident, but is a constant and permanent performance determined in the great majority of cases by the presence of light and to a very limited extent by the character of the substratum. Aeration and humidity are two other factors which also enter into sporophores production on artificial media.

INFLUENCE OF SUBSTRATUM ON CHARACTER OF HYMENIUM

The influence of the host or substratum on the character of the spore-bearing surface is well illustrated in the several strains of *Polystictus hirsutus* when grown in artificial cultures. In all of these strains the best developed and well-defined pore surfaces are produced on carrot, malt, and parsnip agars. On these the pore surface is fairly typical of that produced in nature, both as to size and color of pores. In the prune agar the pore surface is usually reduced in the majority of the tubes to a few scattering irpiciform spines. On some of the prune-agar tubes the spines are not flattened like those of an Irpex, but are round like those of a Hydnum. In the corn-meal tubes the hymenium in a large majority of cases is very similar to that produced in various species of the Thelephoraceae, being reduced to a smooth or slightly granular surface in which there are no definite pores. In all of the tubes (carrot, malt, prune, corn-meal, and parsnip agars) there is an enormous production of spores irrespective of the character of the hymenium. In fact, in some of the tubes the first evidence of any sporophore production is the deposit of spores on the opposite side of the culture tube when even a careful examination with a hand lens fails to show any signs of pores or spines.

The size, shape, and coloring of the pores and tubes produced in artificial cultures on many of the agars are practically identical with those found in nature for a given species. However, it often happens that in abortive sporophores on certain agars the coloring is not as pronounced as on agars where the sporophores reach their full development. In *Polyporus cinnabarinus*, for instance, the sporophores produced on potato agar are nearly white, while the same strain will produce the typical grenadine-red to flame-scarlet pores on malt.

TRUE PILEI IN ARTIFICIAL CULTURES

One of the most interesting facts brought out in this investigation was that in all of the thousands of cultures made with the hundreds of sporophores produced not a single one had a typical pileus, unless the fungus was a gill-bearing form (Agaricaceae), when the culture tubes were so placed that the slant faced the light in such a manner that its rays were

more or less at right angles to the agar surface where the hymenium was being developed.

At first it was believed that the absence of pilei was probably due to the fact that the tubes were left in a horizontal position and therefore pilei had no chance to develop. The same results were obtained when the tubes were placed vertically with the slant side facing the light as when placed horizontally. Very recently, however, the writers have devised a method by which small but otherwise typical pilei have been grown on artificial media in the test tubes. The method was as follows: In place of arranging the culture tubes so that the light fell directly on the slanted surface the tubes were placed in one of two positions (1) vertical in opaque boxes in such a manner that the rays of light would fall on the tops of and parallel to the tubes and none on the sides or bottom, and (2) nearly horizontal, with slanted surface of the agar turned downward but with the light again falling only on the tops and parallel with the tubes. Typical sporophores were produced by this means for *Polyporus dryophilus*, *P. hirsutus*, and *Fomes rimosus*, the only species tried so far. Whether this method will produce pilei with all species is not known. There are objections to both methods where one desires to obtain spores for plating in order to obtain individual spore colonies—viz, when the culture tubes are kept vertically the discharged spores fall on the agar rather than on the inner surface of the culture tubes and when the culture tubes are kept nearly horizontal with the slanted surface downward from the first the mycelium grows around the edges of the agar onto the glass, thereby covering the surface of the tubes where the spores will fall. There is also this further objection to both methods—viz, that the agar in drying separates from the glass tubes in a very irregular manner in place of from only the top surface as it does when the tubes are kept horizontal with the slanted surface uppermost.

INFLUENCE OF LIGHT ON THE FORMATION OF PILEI

The pilei of the Polyporaceae always developed in such a manner that their tops were directly toward the sunlight. Also the pilei of the Agaricaceae when grown in cultures were strongly proheliotropic from the very beginning of their formation. This positive heliotropism was especially marked when the sporophores of *Lentinus lepideus* and *Pleurotus ostreatus* were developing. The writers tried *P. ostreatus* in three different positions: (1) Culture tubes placed vertically but with the lower part so shaded that the light entered at the top of the tube; (2) tubes placed horizontally and covered with black paper so that the light entered only at the top of the tube and this placed toward the light; (3) the third experiment was made in a flask in which the medium was slanted on the side of the flask. The flask was placed upright and the development of the sporophores from this more or less slanting surface was observed. In every instance from the earliest development of the

sporophore to the complete expansion of the pileus, the sporophores always pointed directly toward the light. In the case of the vertical tubes it was to be expected that normally these sporophores would grow vertically, since it was presumed that gravity as well as sunlight might be a factor in the upward growth of the sporophore. When the tube was placed horizontally, a totally different condition existed. In this case the sporophore developed directly toward the sunlight but at right angles to the force of gravity. In the experiment with the flask the upper two-thirds of the flask was covered with dark paper. Ten or fifteen sporophores developed on the slanting surface of the agar in the flask, all of them without exception pointing downward toward the source of light.

Lentinus lepideus was also grown on a more or less vertical slant with the face of the slant turned towards the light. Perfect sporophores which developed under these conditions always turned directly toward the sunlight.

INFLUENCE OF GRAVITY ON THE FORMATION OF PORES

In all of the experiments conducted with the Polyporaceae the formation of the pores was always parallel to the action of gravity. If the tubes were left in their original horizontal position with the slant side upward, pores developed on the slant with their mouths pointing upward, thus making them parallel to gravity. If, on the other hand, the culture tubes were placed in a vertical position, the pores were formed on the sides of the slant in such a manner that their mouths pointed more or less downward, or both upward and downward in some cases and parallel to the force of gravity. In fact, the position of the pores of a fungus which produces a vigorous sporophore in artificial cultures could be governed at will by simply changing the position of the tube in reference to gravity. It seems therefore that while light is usually the main factor governing the initiation of a hymenium, gravity is the dominant force which determines in what direction the pores will point, irrespective of the incident light or whether the spores when discharged will fall onto the walls of the pores or not.

INFLUENCE OF SUBSTRATUM ON PORE FORMATION

Although it is a well-known fact that cultures of strictly parasitic fungi can fruit on a wide range of media irrespective of the special host on which the parasite usually thrives, the idea seems to have been generally accepted that some special culture media would have to be used in order to obtain sporophores of the Polyporaceae on artificial media in test tubes, flasks, etc. The experiments here given show that the same general rule as to food material applies to the wood-rotting fungi as a whole—viz, that no special decoction is necessary for each species in order to obtain sporophores; in fact, the wide range of artificial media upon which many of the Polyporaceae have fruited is rather remarkable when the nature of these fungi is taken into consideration.

Table XVI shows that there were 56 strains of wood-rotting fungi which produced sporophores on malt agar, 52 on corn meal, 48 on prune, 44 on carrot, 27 on parsnip, 14 on potato, 11 on celery, 11 on beet, 10 on bean, and only 1 on alfalfa agar, while there are four of these agars pre-eminently suitable for sporophore production—viz, malt, corn meal, prune, and carrot, in the order given.

POSITION OF SPOROPHORES ON MEDIA

One most interesting fact in reference to sporophore production on artificial media is the fact that a very large percentage, probably more than 95 per cent, of the sporophores were developed on the upper half of the slant. No perfect sporophores have ever been observed by the writers other than on the slant, although the agar in the tubes often dries out sufficiently to leave ample room for the sporophores to develop from the slant to the bottom of the tube over a distance of from 60 to 80 mm. Whether this means that the formation of the sporophore was dependent upon a small amount of moisture or whether it needed the greater aeration which the upper end of the tube afforded is not known. In many instances, especially with the Stereums, the fruiting surface was formed in a narrow zone at the extreme upper limit of the agar slant. The studies so far made indicate that an important factor in this case is probably the drier condition of the agar at the upper end of the slant. It would seem that if the drying of the agar was the only requisite for the formation of sporophores that as the agar dries in the lower portion of the tube a condition would be reached which would normally produce sporophores; yet such is not the case. The by-products produced by the fungus may be more or less deleterious to the formation of sporophores; and, since such by-products would be more abundant in the lower portion of the tube than under the slant, it follows that few, if any, sporophores would develop there.

DENSITY OF MYCELIUM AND SPOROPHORE PRODUCTION

Another very peculiar fact develops that the sporophore production usually occurs on that portion of the agar slant where the aerial growth of mycelium is the least. If a dense mass of mycelium forms over the entire agar slant, the chance for the formation of pores on such a surface is materially lessened. This may explain to some extent why sunlight plays an important part in the production of sporophores, since cultures kept in the dark usually develop a denser mass of mycelium on the surface of the slant than corresponding cultures in the light. There are many species of fungi which have been tried in both light and darkness in which the aerial growth is very limited, even when grown in the dark; and still no sporophores were produced when in darkness, but were produced in the light.

INFLUENCE OF INOCULUM ON SPOROPHORE DEVELOPMENT

In a few of the species of fungi the interesting fact developed that when tissue from sporophores was used for the inocula the presence of this tissue materially shortened the development period for sporophores on the agar. For instance, in *Polyporus albidus* from *Pinus ponderosa* (FP 21875), when tissue was used as an inoculum on corn-meal agar, the development period of the sporophore was 5 days; when mycelium from potato agar was used the development period was 11 days; for *P. anceps* from *Thuja plicata* (FP 21801), when tissue was used on malt the development period for the sporophore was 5 days; when mycelium from potato agar was used for the inoculum on malt agar the development period was 12 days. This rapid formation of the sporophore when tissue was used as an inoculum was characteristic of many of the strains of this group of fungi.

This shortening of the development period of the sporophores is also found occasionally in other species of fungi than this group. For instance, it was rather marked in *Polyporus dryophilus* when fresh sporophore tissue was used, in various strains of *Trametes peckii*, and to a slight extent in *Fomes roseus*. In several cases not only was the development period for sporophores shortened by the presence of pieces of sporophore used as the inoculum, but cultures made from infected wood, mycelium or spores produced sporophores only on one or two agars, while the same species would produce sporophores on several media when the inocula were pieces of sporophores. In those cases where the sporophore tissue shortened the development period, the pores usually but not always start directly on the tissue inoculum and then spread rapidly often over the entire agar slant. In no case was the development of the pores limited to the pieces of inoculum, while in many instances the pores would start on areas not immediately adjacent to the inoculum.

SUMMARY

(1) The following criteria were found of value in the differentiation of the various species: (*a*) Macroscopic characters, including rapidity of growth, color of aerial and submerged mycelium, character of the aerial mycelium as to texture, etc., staining of the agar, decoloration of the agar, the comparative rate of growth between the aerial and submerged mycelium, etc. (*b*) Microscopic characters, such as septation, branching, size and color of hyphæ, clamp connections, polymorphism in spore formation, etc.

(2) The sunlight was found to accentuate the colors and tone down the mycelial growth of the fungus, thereby making it more characteristic and uniform for a given species than when placed under similar conditions in the darkness or in weak diffused light.

(3) The cultural characters of vegetative development of the various strains of a given species of fungus show no appreciable difference between cultures of this fungus whether obtained from infected wood or from

sporophores; neither do the hosts of the fungus seem to make any marked changes in the fundamental cultural characters when strains from different hosts are compared. There may be minor differences due to the host from which the strain came but nothing more.

(4) When the cultural characters of closely related but really distinct species are compared, marked and constant differences in the character of the mycelium will be found on certain corresponding agars in the series of cultures representing the two species, while if the two fungi are really the same species, no constant differences of specific rank will occur. Unknown rots can also be identified by making pure cultures of the causative organism from the diseased wood and determining from the cultural characters of the fungus thus isolated its identity.

(5) The presence of light is essential to the production of sporophores when grown on artifical media in the great majority of fungi here investigated, while the character of the substratum plays only a very minor roll in sporophore initiation.

(6) The medium on which the fungus is grown often governs to some extent at least the form of the hymenium which develops.

(7) The size, shape, and color of the pores and tubes produced in artificial cultures on many of the agars are practically identical with those found in nature for a given species.

(8) The pilei of both the Poplyporaceae and the Agaricaceae when grown in pure cultures on artificial media are from the very beginning of their formation strongly proheliotropic, while the formation of the pore tubes in the Polyporaceae is always such that they are placed parallel to the action of gravity.

(9) In a few species of fungi the presence of tissue as the inoculum shortened the period of sporophore development from one to several days.

(10) Workers with wood-rotting fungi now have the following means for determining the identity of a given fungus or the causative organism of a given rot: (*a*) The sporophore characters as usually found in nature, (*b*) the characters of the rot produced, (*c*) the vegetative characters developed when grown in pure cultures on artificial media when exposed to light, and (*d*) the characters of the sporophores and various spore forms when produced on artificial media.

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GOSSYPOL, THE TOXIC SUBSTANCE IN COTTONSEED

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REVIEW OF PREVIOUS WORK

Since our previous publication (*17*)[1] on this subject, several articles have appeared in which other explanations of cottonseed-meal poisoning have been offered.

Thus, Rommel and Vedder (*14*) have suggested that poisoning by cottonseed meal is similar to beriberi, and is caused by deficient diets. This view was based on the similarity of post-mortem symptoms noted in pigs fed on rice and tankage.

Wells and Ewing (*15*) have concluded that cottonseed-meal injury is due in large part to incomplete diets.

Richardson and Green (*11*) fed white rats and concluded that cottonseed meal and flour are not actively toxic, but contain insufficient minerals and possibly inadequate amounts of the fat-soluble growth-promoting substance.

Osborne and Mendel (*10*) have secured results similar to those of the last-named authors with cottonseed meal and flour, but on subsequently feeding raw cottonseed kernels supplied by us, they have corroborated the results which we had obtained with the kernels. They admit the presence of a deleterious substance in raw cottonseed, but apparently still hold the view that cottonseed meal, the product resulting from cooking the kernels and pressing out the oil, is nontoxic, at least for rats and chickens (*9*).

Inasmuch as no comparative experiments with an isolated and purified substance have been reported, we present the results of additional experiments with various animals to supplement those given in our previous experiments, in which rabbits and fowls were used.

The toxic effect of an ether extract of raw cottonseed has been well shown in the rat-feeding experiments described by McCollum and coworkers (*6*) and by Osborne and Mendel (*10*). This extract contains about 2 per cent of gossypol, which is equivalent to about 0.6 per cent of the weight of the kernels from which the extract is obtained. Our rat diet, containing 20 per cent of this extracted oil, caused prompt decline in grown rats. Osborne and Mendel (*10*) used as little as 1 per

[1] Reference is made by number (italic) to "Literature cited," pp. 100–101.

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cent of the extract (equivalent to about 0.02 per cent of gossypol in the diet) and found that the growth of the rats was greatly retarded. Our experiments have led to the conclusion that raw cottonseed kernels are highly toxic to rats, but that cooked cottonseed is only slightly toxic. Whether cottonseed meal made from cottonseed sufficiently cooked with moist heat is toxic to rats seems to depend on the diet in which it is fed. In a short feeding experiment a diet such as was used by Richardson and Green (*11*) (45 per cent of cottonseed meal, 17 per cent of whole milk powder, 10 per cent of starch, and 28 per cent of lard) has shown no definite toxic effect [1] on our rats even when it contains a short-cooked (28 minutes) meal. When the meal is the sole source of vitamines, protein, and minerals, we have rarely had such favorable growth as is reported by Richardson and Green (*11*) and by Osborne and Mendel (*10*).

If compared with ether-extracted raw cottonseed or with soybean meal, the rate of growth has been very small. The explanation of this, according to Osborne and Mendel, might be that the diet was unpalatable and that consequently less food was ingested. Unpublished experiments indicate to us that there still remains something toxic in long-cooked cottonseed meals which in restricted diets is objectionable to rats, causing a lower food intake, but the effect of which is overcome in supplemented diets.

This seems to be the same phenomenon discussed by McCollum (7), who finds that in diets very well supplemented the toxic effect of the fat of wheat embryo and other slightly toxic substances is overcome. The evidence of a toxic factor of moderate intensity for rabbits and pigs is also furnished in our rabbit and pig experiments.

TOXICITY OF RAW COTTONSEED KERNELS

In a large number of experiments with rats we have found that the effect of cottonseed products on rats can be predicted accurately when chemical tests indicating the presence or absence of gossypol have been made (Table I). In order to appreciate the significance of some of the experiments we may briefly describe the properties of gossypol. A yellow plant pigment having the apparent formula $C_{30} H_{28} O_9$ (molecular weight 532), not soluble in and not extracted by petroleum ether, readily soluble in acetone and ether, moderately soluble in alcohol, benzene, and chloroform, dissolves readily in sodium hydroxid (NaOH) and sodium carbonate (Na_2CO_3) and is slowly soluble in sodium bicarbonate ($NaHCO_3$). It may be titrated as a dibasic acid with aqueous alkalies. It crystallizes well from a mixture of ether and acetic acid as a sparingly soluble substance containing 10.1 per cent (1 molecule) of

[1] Data in article not yet published.

acetic acid. When dissolved in ether or oil and treated with anilin, it forms a bright-yellow insoluble substance which is apparently a dianilin salt of gossypol (1 molecule of gossypol to 2 molecules of anilin) $C_{30}H_{28}O_9$, $2C_6H_5NH_2$. This substance is very insoluble in most solvents, even aqueous sodium hydroxid.

TABLE I.—*Results of feeding raw cottonseed kernels in milk diets to rats*

Diet.	Rat No.	Weight.			Period.	Remarks.
		Initial.	Final.	Change.		
		Gm.	*Gm.*	*Per cent.*	*Days.*	
Diet 366 (59 per cent of kernels, 17 per cent of whole-milk powder, 10 per cent of starch, 14 per cent of lard).	43	133	110	−17	7	Died 11th day.
Do	44	217	179	−17	7	Discontinued.
Do	45	156	128	−18	7	Recovered on ether-extracted kernels.
Do	46	139	109	−25	7	Do.
Diet 377 (30 per cent of kernels, 38 per cent of milk-powder, 11 per cent of starch, 14 per cent of lard, 7 per cent of butter).	71	154	100	−32	18	Died.
Do	72	186	112	−40	21	Do.
Diet 378 (28 per cent of dry-heated (110° C.) kernels, equivalent to 30 per cent of raw, 40 per cent of milk-powder; otherwise like diet 377).	74	130	87	−33	23	Do.
Do	75	117	80	−31	19	Do.
Diet 379 (10 per cent of kernels, 50 per cent of milk-powder, 12 per cent of starch, 28 per cent of lard).	58	160	107	−33	61	Do.
Do	59	170	112	−34	61	Do.
Do	60	131	103	−21	59	Discontinued.
Control diet 364 (60 per cent of milk-powder, 12 per cent of starch, 28 per cent of lard).	60	105	122	+16	20	Do.
Diet 367 (39 per cent of ether-extracted kernels, 17 per cent of milk-powder, 10 per cent of starch, 34 per cent of lard).	47	112	185	+65	85	Subsequently declined from disease.
Do	48	184	188	+ 2	85	Do.
Do	45	128	177	+38	14	Were on unextracted kernels for 1 week previous.
Do	46	104	159	+53	14	Do.

TABLE I.—*Results of feeding raw cottonseed kernels in milk diets to rats*—Continued

Diet.	Rat No.	Weight.			Period.	Remarks.
		Initial.	Final.	Change.		
		Gm.	*Gm.*	*Per cent.*	*Days.*	
Diet 368 (20 per cent of ether-extract(equivalent to about 60 per cent of raw kernels), replacing 20 per cent of lard in control diet 364).	51	139	112	−19	6	Died.
Do	52	125	101	−19	8	Discontinued.
Do	53	206	185	−10	8	Do.
Do	54	135	118	−12	8	Do.
Diet 369 (0.4 per cent gossypol (equivalent to about 20 per cent of ether extract), replacing 0.4 per cent of starch in control diet).	29	140	107	−23	8	Do.
Do	30	113	94	−16	4	Do.
Do	31	162	144	−11	4	Do.
Do	32	133	106	−20	7	Died.
Diet 373 (0.1 per cent of gossypol added to control diet).	58	141	102	−28	112	Discontinued.
Do	59	80	71	−11	108	Died.
Do	60	81	70	−13	112	Do.
The survivor, rat 58, was then put on the same diet minus gossypol.	58	102	138	+36	21	Discontinued.

The sparingly soluble compounds of gossypol with acetic acid and with anilin have been used to estimate the amount of gossypol present in cottonseed kernels. Both methods have given results which show that gossypol exists in cottonseed kernels to the extent of approximately 0.6 per cent.

In order to explain the change in toxicity in cottonseed after being cooked in the mill, we offer the following hypothesis: Under the action of moist heat the gossypol streams from the glands and is spread over the seed tissue. Part is oxidized to a less toxic substance which we may for convenience call "D-gossypol;" part is left in combination with the bases or protein as a salt of gossypol; and part is expressed in the oil. The degree to which these changes take place is dependent on the method of cooking and the condition of the seed. In dry heating to 100° C. there is practically no decomposition of the gossypol. In very dry seeds the gossypol may not spread over the seed tissue and be changed unless much moisture is added and the cooking prolonged.

Some quantitative data on the amount of gossypol left in the seed have been obtained from samples of kernels cooked various lengths of time—5, 10, 20, and 28 minutes. The percentage of gossypol extracted by ether in these cases was, respectively, 0.62, 0.24, 0.10, and 0.07.

METHOD OF REMOVING THE TOXIC SUBSTANCE FROM THE ETHER EXTRACT

By treatment of the ether extract of raw kernels with an excess of anilin the gossypol is practically quantitatively precipitated. The dianilin salt produced is extremely insoluble in most solvents except hot anilin and alcoholic potassium hydroxid (KOH). The substance itself is not toxic because of its insolubility. It passes through the alimentary canal unchanged, as can be seen by a glance at the feces. One-half gm. doses of this anilin compound were fed for seven consecutive days to a rabbit without result, and it was also given to rats in a milk diet (0.3 per cent). The food intake of the rats was not diminished; nor were the rats affected perceptibly

Gossypol "acetate" was then prepared from this compound as follows: The substance was decomposed by means of an alcoholic alkali. The anilin was steamed off, and the gossypol was extracted with ether and crystallized as the "acetate" by the addition of acetic acid. This was fed in amounts (0.25 per cent) equivalent to the anilin compound (0.3 per cent) fed previously. The rats which had not been affected by the anilin compound were promptly affected and consumed but little food (see Table II).

By passing steam through the extract from which the anilin compound had separated the excess anilin was removed, and the resultant oil did not prove toxic to rats. The result of this experiment has led us to believe that gossypol is the only substance in raw cottonseed possessing marked toxic properties. This conclusion was indicated in our previous experiments (*17*), wherein we found that the gossypol extract freed from gossypol was not toxic.

TABLE II.—*Results of feeding gossypol "acetate" to rats*

Diet.	Rat No.	Weight.			Period.	Remarks.
		Initial.	Final.	Change.		
		Grams.	*Grams.*	*Per cent.*	*Days.*	
Diet 436 (gossypol "acetate" (0.25 per cent) prepared from decomposition of the insoluble nontoxic gossypol-anilin compound, added to the control diet).	201	166	119	−28	12	Died.
Do.	202	122	91	−25	12	Do.
Do.	203	128	94	−26	14	Do.
Do.	160	117	79	−32	14	Do.
Diet 448 (12.5 per cent ether extract freed from gossypol by treatment with anilin, replacing 12.5 per cent of lard in control diet).	171	150	190	+27	39	Alive.
Do.	175	155	190	+25	39	Do.
Do.	94	95	118	+24	39	Do.
Do.	96	124	167	+35	39	Do.
Do.	90	122	137	+12	39	Do.

TOXICITY OF GOSSYPOL TO RABBITS

In our previous paper (*17*) most of our feeding experiments with gossypol were with the "acetate," a crystalline substance containing acetic acid in its composition. This product had the same toxic action as the product precipitated by petroleum ether; therefore we inferred there was no change wrought by crystallization. We have recently fed gossypol in amounts equivalent to a toxic weight of cottonseed kernels and have found it to produce serious results in every case. Where gossypol itself is added to a diet in appreciable amounts, the toxic effect is marked.

Gossypol was mixed with the feed in four forms: (1) Precipitated (by petroleum ether), (2) recrystallized "acetate" (10.1 per cent of acetic acid), (3) "free" gossypol, a very pure product, and (4) as the sodium salt of gossypol (gossypol "acetate" neutralized with three molecules of sodium hydroxid, 10.5 c. c. of *N*/2 alkali to 1 gm. of substance). The results are summarized in Table III.

TABLE III.—*Results of feeding gossypol to rabbits*

Diet.	Rabbit No.	Weight.			Quantity of gossypol eaten.	Equivalent in extracted kernels.	Feeding period.	Result.
		Initial.	Final.	Gain or loss.				
		Grams.	*Grams.*	*Grams.*	*Grams.*	*Grams.*	*Days.*	
Gossypol precipitated from ether solution by petroleum ether.	990	1,490	1,205	−285	1.81	200	24	Died.
Do.	996	2,440	2,105	−335	2.61	290	26	Do.
Do.	998	1,075	850	−225	.68	75	14	Do.
Recrystallized gossypol "acetate."	994	2,330	2,180	−150	.87	97	14	Do.
Do.	1,001	975	820	−155	.47	53	8	Do.
Gossypol free from acetic acid (0.1 gm. daily per animal, equivalent to about 17 gm. of raw cottonseed kernels).	21	2,000	1,900	−100	0.4	....	4	Died 15th day.
Do.	22	1,500	1,440	− 60	.33	....	4	Died.
Gossypol "acetate"	17	950	800	−150	.433	....	....	Do.
Do.	18	850	750	−100	.333	....	....	Died 11th day.
Sodium salt of gossypol	19	700	590	−110	.153	....	....	Died 13th day.

A peculiar feature about the effect of gossypol and oftentimes of cottonseed kernels is that the animals may eat these substances for several days without being affected, then they may suddenly cease eating, waste away, and finally die. This was the case with rabbits 21 and 22 in this experiment.

In our previous paper (*17*) we described the nontoxic product obtained by oxidation of gossypol by action of air on its alkaline solution. This oxidation product may also be formed to some extent in the cooking of cottonseed, but there is no evidence of it. The meal still contains con-

siderable amounts (about 1 per cent) of a substance which we have called "D-gossypol."

"D-gossypol" is very slightly soluble in ether. For rabbits ether extraction does not render the meal nontoxic. But where we find in the meal after 6 hours' extraction with ether considerable amounts of a substance giving color reactions for gossypol or "D-gossypol," it would seem that the substance is bound in some way. To explain this we have assumed that it may be combined with the protein or some other constituent. There is some evidence of this in the properties of these substances. We have mentioned that gossypol combines with anilin and with acetic acid to form less-soluble compounds. Possibly similar combination may take place with free amino and free carboxyl groups in the protein molecule. Marchlewski (*8*) mentioned the fact that gossypol behaves like tannin toward basic dyes. Tannin also precipitates proteins as insoluble compounds. A similar combination of gossypol with protein may occur in the cooking of cottonseed.

Confirmatory evidence that these substances may be the cause of cottonseed-meal poisoning is given in the previous publications of this Station. Thus, when cottonseed meal is treated with an alcoholic alkali (*19*), the meal is rendered nontoxic to rabbits. When the meal is fed with iron salts to pigs (*18*) and rabbits (*16*), the toxic effect is greatly diminished. Pigs fed on cottonseed meal and corn meal (1 : 3) with ferrous sulphate (*copperas*) did not die in 180 days, whereas without ferrous sulphate all the animals died. Rabbits were fed 106 days with ferric ammonium citrate without harmful results. We have explained this by assuming that the alkali treatment promoted oxidation of the gossypol and by assuming that the iron salt formed an insoluble precipitate with the gossypol, or possibly assisted the organism to oxidize it.

The experiments referred to in this article support our previous view that gossypol is toxic and that it is the only toxic substance in the raw kernels.[1] Extensive experiments with various meals with rats, rabbits, fowls, and swine show that there still remains, even in thoroughly cooked meals, an injurious factor. Such thoroughly cooked meals are harmful to rabbits and swine, but seem to have little effect on rats and fowls when fed on adequate diets.

PRELIMINARY EXPERIMENTS WITH PIGS

In order further to test the correctness of our view that cottonseed-meal injury is due to a toxic substance rather than to dietary deficiencies, we have conducted a few preliminary experiments with small pigs.

It seemed desirable, in view of the extreme position taken by Rommel and Vedder (*14*) to ascertain (1) whether gossypol is toxic to pigs; (2)

[1] The results of other experiments showing that cooking exerts a profound influence on the toxicity will be published at an early date.

whether extraction of gossypol from cottonseed by a solvent renders the residue nontoxic; (3) whether by the addition of vitamine-containing feeds, cottonseed-meal poisoning can be averted.

Four small Duroc-Jersey pigs were confined in small pens about 3.5 by 8 feet. The pens had a concrete floor which was bedded with pine shavings. The water used was secured from the city mains. The pigs were fed the diets given in Table IV.

TABLE IV.—*Percentage composition of diets for pigs*

Feed.	Pig 1.	Pig 2.	Pig 3.	Pig 4.		
				Period 1 (1st–28th day).	Period 2 (29th–38th day).	Period 3 (39th–45th day).
Cottonseed meal	25			21.7	30	45.5
Corn meal	75	49.75	75			45.5
Gossypol		0.25				
Wheat bran		50		65	60	
Ether-extracted cottonseed kernels			25			
Milk (solids)				13	10	9
Green feeds				([a])	([a])	([a])

[a] About one-half pound daily.

Pig 2 received at the start 1.22 gm. of gossypol daily. This figure is based on yields (about 1 per cent) of crude crystalline gossypol acetate, obtained from "oil-free" cottonseed kernels. Gossypol "acetate" equivalent to the required amount of gossypol was dissolved in ether; the acetic acid present was removed from the ether solution by agitation with water. The ether solution of gossypol was then spread over a part of the corn meal and the ether evaporated. This was no doubt an unnecessary procedure, as we have found no difference in the action of the "acetate" and the "free" gossypol.

Pig 3 was fed on cottonseed kernels from which practically all the gossypol had been removed by percolation with ether.

Pig 4 was fed with a view to supplying any deficiency of vitamines in the cottonseed meal by wheat bran, whole milk, and some green food, chiefly leguminous. It is not possible to give the exact composition of the ration of this pig. The green feed (about ½ pound daily) was not always consumed. By disregarding the green feed eaten and by assuming that the milk contained 12 per cent of solids, the composition of the diet was approximately as given above.

Figures 1 and 2 and Tables V to VII summarize the important data of the experiment.

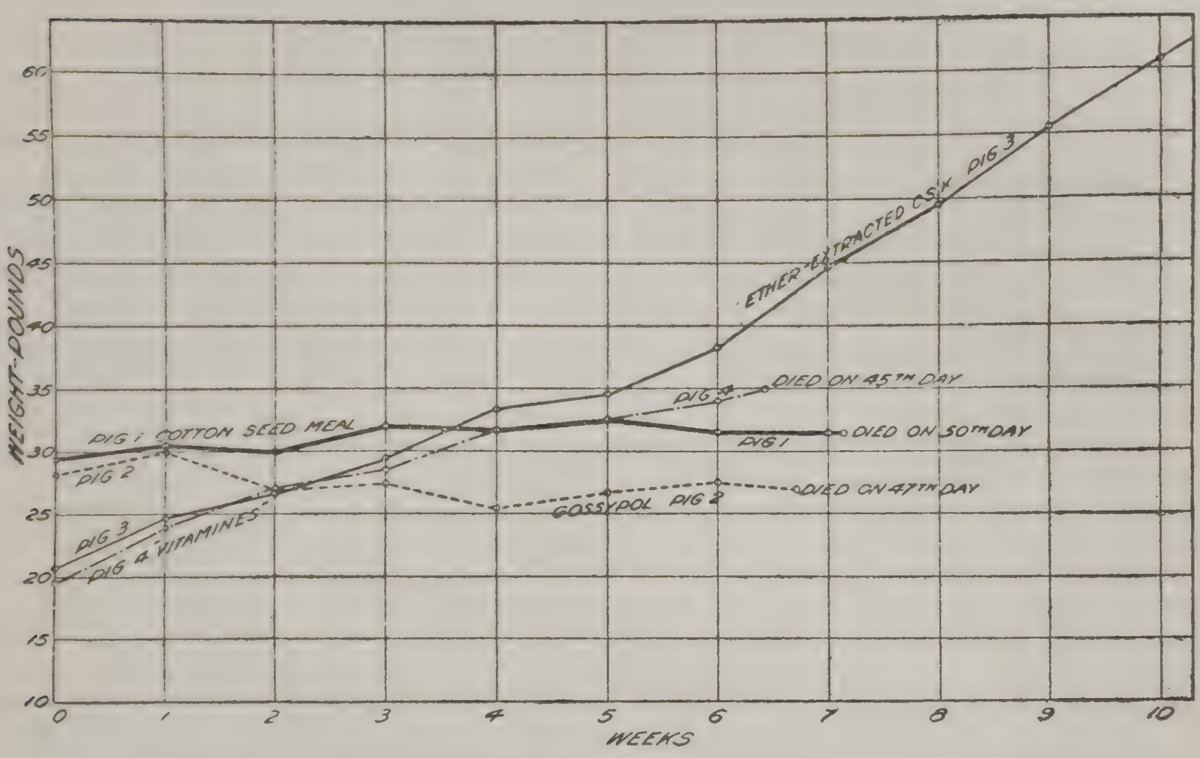

FIG. 1.—Graphs of the growth of pigs 1, 2, and 3. "C. S. K."=cottonseed kernels.

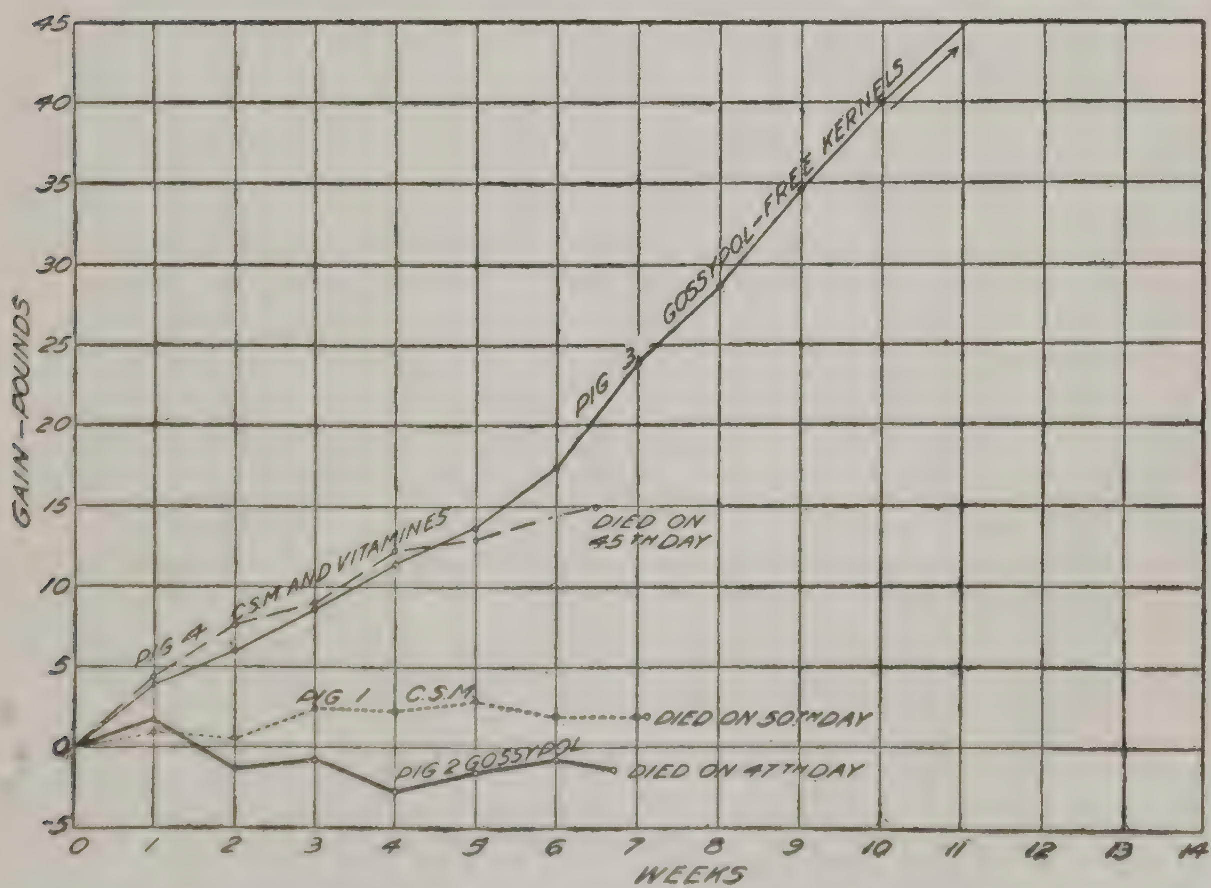

FIG. 2.—Graphs of the gains per week of pigs 1, 2, and 3. "C. S. M."=cottonseed meal.

TABLE V.—*Results of feeding various diets to pigs*

Pig No.	Feed.	Weight.			Result.
		Initial.	Final.	Gain.	
		Pounds.	*Pounds.*	*Pounds.*	
1	Cottonseed meal	29.5	31.5	2	Died 50th day.
2	Gossypol	28.25	27	−1.25	Died 48th day.
3	Ether-extracted cottonseed kernels	20.75	[a] 44.75	24	Lived.
4	Cottonseed meal, milk, etc	19.5	35	15.5	Died 45th day.

[a] Weight of pig 3 on the 50th day. This pig weighed 128 pounds when the experiment was discontinued on the 155th day, a daily gain of 0.69 pound.

TABLE VI.—*Weight of cottonseed feed consumed, by weeks* [a]

Week.	Pig 1 (cottonseed meal).	Pig 2 (gossypol).[b]	Pig 3 (extracted cottonseed kernels).	Pig 4 (cottonseed meal).
	Pounds.	*Gm.*	*Pounds.*	*Pounds.*
1	1.68	8.37 (1.82)	1.3	1.3
2	1.7	9.76 (2.15)	1.5	1.52
3	1.63	8.04 (1.77)	1.75	1.43
4	1.58	6.76 (1.50)	2.18	1.62
5	1.36	4.06 (.90)	2.18	2.14
6	1.27	5.56 (1.22)	2.63	2.8
7	1.04	3.50 (.77)	2.53	1.1
Total cottonseed feed eaten	10.26	49.95 (10.13)	14.07	11.91
Total feed eaten	41.0	40.5	56.3	46.0

[a] Maximum estimates are given for pigs 1, 2, and 4.
[b] Figures in parentheses give the weight (in pounds) of oil-free kernels, which correspond to the gossypol eaten.

TABLE VII.—*Comparison of post-mortem notes on pigs 1, 2, and 4*[a]

Organ, etc.	Pig 1.	Pig 2.	Pig 4.
Lungs	Congested, edematous.	Congested, edematous.	Extremely edematous, with some congestion.
Heart	Thrombus		Thrombus.
Chest cavity	2 to 3 ounces of serous fluid.	4 ounces of fluid	About 16 ounces of fluid.
Abdominal cavity	Slight excess of fluid.	Slight excess of fluid.	Slight excess of fluid.
Small intestines	Considerable injection of blood vessels.	Deeply injected	Inflamed areas.
Penis	Sheath swollen	Sheath swollen, organ paralyzed, and protruding.	Sheath swollen.
Nutrition	Poor	Very poor	Good.

[a] These pigs were examined by Dr. G. A. Roberts, Veterinarian of this Station.

GENERAL DISCUSSION

For the first few days of the experiment all the pigs ate well, and all gained in weight. Pigs 1 and 2 occasionally left part of their feed. In two weeks' time all except pig 3 began to show loss of appetite and regularly left a portion one-half to one-fourth of their feed. On the twenty-fifth day the pig 2 (fed gossypol) was quite sick and not able to walk well. At this time pig 3 was the thriftiest of the four, while the rations of No. 2 and 3 were reduced on account of refusal to eat. On the twenty-ninth day the ration of pig 4 was changed to 1 part of cottonseed meal and 2 parts of bran. On the thirty-second day the feed of pig 2 was changed. The wheat bran was replaced by middlings, for which the pig had a better appetite. On the thirty-ninth day the wheat bran in the ration of pig 4 was replaced by corn meal, the pig getting equal parts of cottonseed meal with corn meal. At that time this pig was leaving one-half to two-thirds of the wheat-bran mixture. For three or four days he ate the new mixture with much better appetite, but then refused a large part and died on the forty-fifth day. Pig 3 maintained perfect appetite up to the forty-ninth day, when she did not clean up the last trace of feed as usual. When removed to the yard to be photographed, it was noted that she had an abnormal gait in walking, the forelegs showing a tendency to double under her. This animal seemed to have a great desire to eat dirt, manure, etc. However, on being allowed the freedom of a large lot, the animal soon recovered. She was given a little ferrous-sulphate solution, chalk, and milk on the fiftieth day. Her normal appetite returned, and in three or four days she was able to trot. At no time did this pig show the rough coat and lack of appetite that characterized the others. On the fifty-second day the feed of cottonseed was increased slightly, and the pig received about one-half pint of milk daily for the following nine days. Whether this pig was suffering from deficiency of some sort in the ration, from lack of exercise, or from the daily intake of a small amount of gossypol in the kernels, we are not able to say. Even granting that this pig had a slight attack of beriberi, we can reasonably conclude from the experiment that the deficiency factor is one quite secondary to the toxicity factor. Plate 1 shows the condition of these pigs at various periods of the experiment.

This preliminary experiment was originally planned to run for a short period, but as the pig on the extracted kernels seemed to be in a pathological condition, in that the forelegs tended to double under her when she attempted to run, it seemed desirable to continue the animal on this diet. At this time, when pig 1, which had been given the diet most closely resembling that of pig 3 in chemical composition, had died in a rather emaciated condition, pig 3 was a plump, very-well nourished animal (Pl. 1, B). It is quite possible that the above-mentioned condi-

tion was due both to a slight toxicity of the extracted kernels and to the restricted diet. This condition was remedied, as previously described. The animal was continued on the outdoor turf lot for 35 days and then removed to a small indoor pen, where for 70 days longer she gained steadily.

Throughout the experiment the daily feed was maintained at 1 per cent (of body weight) of extracted kernels plus 3 per cent of corn supplement.

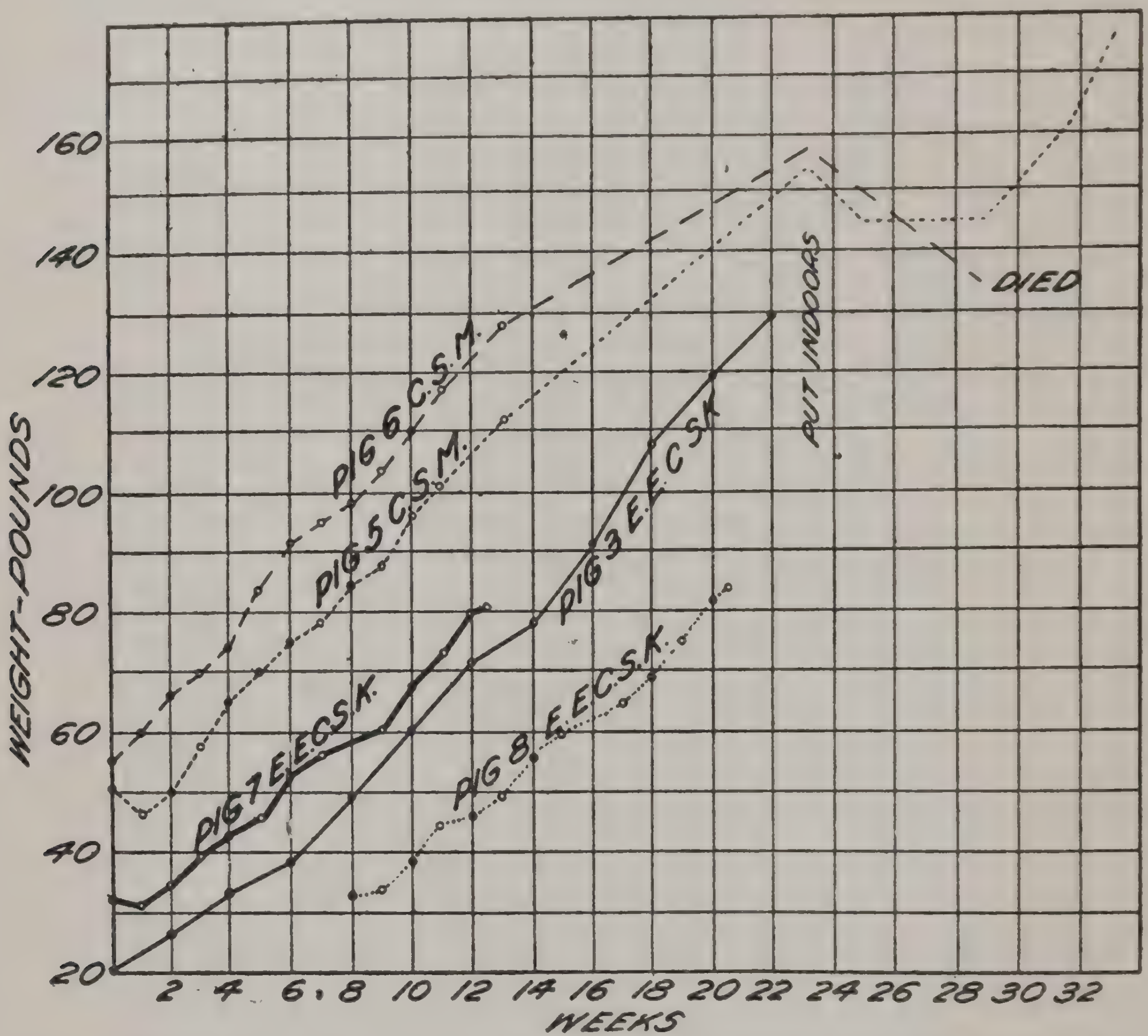

FIG. 3.—Graphs of the growth of pigs 3, 5, 6, 7, and 8. "C. S. M."=cottonseed meal: "E. E. C. S. K."= ether-extracted cottonseed kernels.

Two other small pigs, No. 7 and 8, were also fed on this diet of extracted kernels and corn meal on the same basis as in the previous case. On a few days it was necessary to use whole or cracked corn in place of corn meal, owing to a lack of meal. The results in general were the same. There was fair growth, but after six or eight weeks the pigs lost their keen appetites and developed a tendency to squat on their hindquarters and to walk stiffly. The growth curves in figure 3 show the general results. The gain was fair, 0.581 pound per day for the female and 0.556 pound for the male pig. The experiment was discontinued on the eighty-eighth day.

The female pig was observed for a few weeks after the experiment. The animal continued to increase in weight, but still retained the squatting tendency. Addition of small amounts of milk and outdoor exercise did not eliminate this condition. The nutrition of the animal was excellent throughout.

In view of the slight toxicity of the extracted kernels, as shown in our rabbit experiments, it is possible that this slightly pathological condition may be due to a toxic factor, although with such very young pigs it may be due to the limitations of this food mixture.

No doubt if these pigs had been continued on this diet confined to pens, they would have ultimately failed, as do swine fed on restricted diet of cereal grains (see Hart and McCollum, *2*). This phenomenon, however, should not be confused with what is commonly understood as cottonseed-meal "injury" or poisoning.

We do not claim, however, that this diet is an adequate one, and it is quite possible that the condition described was due in large part to the inadequacy of certain dietary factors. Just what factors are insufficient in this particular diet is not at present apparent. In considering this question we have taken the view, tentatively, that the supply of vitamines in cottonseed is similar to that of other seeds and that the mineral content is very much better.

The fact that cottonseed is cooked, and subsequently pressed, raises the question, Are the vitamines thus rendered partially inactive or removed by the crude oil? In answer to this, we may point to the excellent growth of rats reported by Richardson and Green (*11*, *12*, *13*,) and Osborne and Mendel (*10*) as evidence that even the cooked meal is as well supplied with vitamines as any similar vegetable food.

With the dietary factors more favorable, as in the cottonseed-meal, experiment to be described, probably the pigs would not have manifested this stiffness of gait and squatting tendency. It is also of interest to compare the mineral content of a diet of cottonseed meal and corn meal (1 :3) with the ash content of a diet found successful for growth in rats. (See Table VIII.)

TABLE VIII.—*Average mineral content of a diet containing 1 part of cottonseed meal and 3 parts of corn meal; also the analysis of minerals of other materials*

Feed.	Protein.	Ash.	Sodium.	Potassium.	Calcium.	Magnesium.	Chlorin.	Phosphorus.	Sulphur.
Cottonseed meal......	36	7.0	0.26	1.66	0.27	0.55	0.04	1.35	0.49
Corn meal...........	87	.8	.113	.19	.015	.122	.07	.264	.20
Average mixture 1:3.	15.5	2.3	.14	.55	.08	.22	.055	51	.20
Pig and rat diet A (*2*).	......	3.3	.022	.335	.266	.275	.041	.248	.089
Diet B (*3*)...........	......	.63	.029	.076	.080	.009	.057	.186	.141
Dry skim milk (*1*)....	......	7.17	.488	1.27	1.34	.146	.935	.979	.357

Rat diet A represents the mineral content of a diet which was found successful for growth of pigs on artificial rations by Hart and McCollum (*2*).

Rat diet B represents the mineral content of a diet which did not produce pronounced stunting in rats (*4*). The diet was satisfactory in other factors.

The recent work of Hogan (*3*) and of McCollum, Simonds, and Pitz (*5*) indicates that the deficiencies of corn lie in the poor ash content, in poor proteins and low amounts of protein, and inadequate amounts of fat soluble A.

Since our pigs on ether-extracted kernels plus corn meal made much better gains than could be expected on whole corn alone under these conditions, our opinion is confirmed that the addition of this cottonseed feed to a corn diet furnishes a greatly improved protein and a mineral basis for nutrition. It is then evident that, where death ensues or poor growth is manifested, this is a result of an injurious substance rather than of dietary deficiencies.

FURTHER EXPERIMENTS WITH GOSSYPOL

Gossypol was fed to two other pigs of approximately 50 pounds' weight. In one case a rather large amount was given in the ration. Pig 5 ate a slop made of 1 pound of a mixture of corn meal and soybean meal (1:3) containing 4.5 gm. of gossypol. The pig showed a poor appetite for the same amount given the next morning, but ate it slowly after a pint of milk was poured into it. Next, a half dose (containing 2.3 gm.) was offered, one-half of which was refused. For the next five days the pig was offered smaller doses, but refused it all or in part, even when tempted to eat by using bran and milk in the feed. The pig showed a good appetite for other feed that did not contain the gossypol mixture. On the fifth, sixth, and seventh days the pig showed no appetite at feed time but seemed sluggish and showed a desire to lie down. The pig lost in weight. The experiment was then stopped. A control pig, No. 6, fed on a similar ration without gossypol showed a good appetite and developed no such symptoms.

After a rest of six days, the previous control pig, No. 6, was used for a gossypol experiment, and No. 5 served as the control. Gossypol was fed in amounts equivalent to that in 0.5 to 0.6 pound of oil-free cottonseed kernels, approximately 2.2 gm. daily. An ether solution of gossypol was dried on corn meal and this was mixed with more corn meal and wheat bran. The other pig received the same ration without gossypol. The gossypol pig ate practically all its feed for a week, and then began to show a poor appetite for it and refused part. On the eighth day one-half the feed was left. On the ninth and tenth days the pig ate scarcely any feed. From the eleventh to the fifteenth day the animal was given dry recrystallized gossypol acetate (10 per cent acetic

acid) as a finely crystalline powder mixed with the feed in place of gossypol evaporated on corn meal. The pig ate it for one day, and then refused it as before. By using corn meal only the animal ate one more feed, but refused it when repeated. During the last few days of the experiment the gossypol animal seemed to be growing weaker and very preceptibly thinner. In the last week the animal lost 3 pounds, while the control gained 2 pounds. This experiment was discontinued on the fifteenth day because of the refusal of the animal to eat.

In both of these cases of feeding gossypol it was very evident that the animals were physiologically affected at an early date. All told not over 15 gm. of gossypol were eaten in the first case and not over 22 gm. in the second case. These amounts of gossypol are equivalent to about 4 and 6 pounds of oil-free cottonseed kernels, respectively.

Besides the direct proof of the existence of a toxic substance in cottonseed meal, we have a strong argument against the deficiency theory in the results of feeding rabbits on cottonseed meal treated with boiling alcoholic alkali. This treatment, which would be expected to destroy the natural vitamines present, so changes the meal that it becomes nontoxic to rabbits. This change in toxicity we have shown is explained by the ease with which gossypol undergoes oxidation in alkaline solution. This was confirmed by feeding to rabbits the products formed by oxidation of gossypol in alkaline solution by air.

CAN COTTONSEED-MEAL POISONING BE OVERCOME IN A FAVORABLE DIET UNDER FARM CONDITIONS?

The two largest and oldest pigs (No. 5 and 6) were fed in a turf lot about 50 feet square. Grass was abundant, and a good part of the time there was water in the lot from frequent rains which also kept the turf soft. These pigs were fed on a mixture of equal parts of cottonseed meal, corn meal or corn, and wheat bran, with a pint of milk apiece each day. The cottonseed meal fed each day was about 1.33 per cent of body weight, rather higher than has been the practice at this Station. It was thought that for a while, about the fiftieth day, the pigs acted somewhat suspiciously. One showed a lack of appetite for the mixture. Its eyes seemed partly closed and somewhat watery. The pigs also seemed rather short-winded. They retained, however, perfect control of their limbs and were able to run very well at all times. Finally, after 160 days of high feeding of cottonseed meal, these pigs were put under cover in a small pen. The pigs were soon eating sparingly and losing weight. The pig which had acted somewhat queerly at times in the experiment became sick and died on the one hundred and ninety-eighth day, showing typical symptoms, although there was also a pneumonic appearance of the lungs. The experiment with the other pig was then discontinued. On changing this pig's feed to corn, he began to regain weight. This animal was later fed corn, wheat bran, meat scraps, etc. (Table IX.)

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TABLE IX.—*Results of feeding cottonseed meal to pigs 5 and 6 under favorable conditions.*

Time of weighing.	Weight.			
	Pig 6.	Pig 5.	Gain per day.	
			Pig 6.	Pig 5.
Day.	*Pounds.*	*Pounds.*	*Pound.*	*Pound.*
Initial (June 28)	55.5	50.5		
92	129	112.5	0.8	0.67
160	158	153.5	.64	.65
177	150	145		
192	142	145		
198 (Jan. 12)	[a] 135	([b])		
217		158		
225–230		[c] 177		

[a] Dead. [b] Discontinued feeding cottonseed meal. [c] Slaughtered.

As long as these pigs were kept in the outdoor lot, no marked symptoms of cottonseed-meal poisoning were noted. It was noted that when kept indoors they lost both appetite and weight.

It is evident from our indoor and outdoor experiments that the effect of cottonseed meal is more severe on pigs kept in pens, a fact that has long been known; however, past records show that typical sudden deaths from "acute cottonseed-meal poisoning" may also occur among pigs receiving cottonseed meal when on pasture. Such deaths may follow excellent gains and may be without previous sickness, often occurring when the animals are exercised violently. Consequently, a conclusion that the meal was without effect during the outdoor experiment can not be drawn. Certainly the subsequent loss in weight after removal from the turf is suspicious. It is plausible to suppose that the outdoor conditions stimulate metabolism so that the animal is enabled to overcome or resist the injurious factor. Possibly the difference in effect of cottonseed meal on rats and pigs may be in part explained by the more vigorous metabolic activity of the smaller animal.

In a series of three recent articles, Richardson and Green (*11, 12, 13*) have well shown the high nutritive efficiency of cottonseed meal and flour for rats, indicating the economic value of this substance. In some points, however, we believe that they have misinterpreted facts. Thus, they speak of the flour as a "highly milled" or "refined" product and account for the apparent slight nutritive superiority of the unbolted meal over the flour by stating that—

> This suggests a greater amount of the growth-promoting substance associated with certain fats in the less highly milled product.

It seems hardly possible in the case of cottonseed meal to effect such a change in nutritive value by mechanical means. We would suggest that the difference was accountable on the basis of different conditions

in the cooking of the products, as we have found these to be the greatest cause of variation in toxicity of meals.

Richardson and Green (*11, p. 316*), state in conclusion:

> Our results indicate that cottonseed meal does not contain sufficient minerals for growth, is not actively toxic, contains efficient protein and perhaps fat-soluble, growth-promoting substances, similar to those of butter fat, but in less adequate quantities.

Our own extensive unpublished experiments on the toxicity of cottonseed products indicate that the toxicity of cottonseed meals varies with the conditions of cooking the raw seed. While we find that the flour and thoroughly cooked meals have no apparent toxicity for rats when fed in diets supplemented by milk powder, these same products fed in unsupplemented diets are inferior to ether-extracted cottonseed kernels. Even thoroughly cooked cottonseed meals are definitely injurious to rats and pigs.

The ash analysis of cottonseed flour given by Richardson and Green (*11, 12, 13*) in each of these three articles differs radically in some respects from that given by Forbes (*1*) (Table X).

Table X.—*Ash analyses of cottonseed flour*

Constituent.	Analysis of cottonseed flour according to Richardson and Green.	Constituent.	Analysis of cottonseed meal according to Forbes.
	Per cent.		*Per cent.*
Inorganic salts	5.50	Ash	7.629
Silicic oxid (SiO_2)	0.14		
Chlorin	None.	Chlorin	.042
Sulphur trioxid (SO_3)	.06	Sulphur	.536
Phosphorus pentoxid (P_2O_5)	2.57	Phosphorus	1.479
Potassium oxid (K_2O)	2.01	Potassium	1.81
Calcium oxid (CaO)	.26	Calcium	.291
Magnesium oxid (MgO)	.25	Magnesium	.599
Sodium oxid (Na_2O)	None.	Sodium	.283

Richardson and Green's data are from an analysis of material left after ignition, which, as is well known, causes loss of elements, such as sulphur and chlorin. While the elements chlorin and sodium are not necessary for plant growth and the amounts present may vary, it seems hardly possible that they are entirely lacking in cottonseed flour. They are certainly essential to animals. It may be noted that the rats of Richardson and Green "have grown and maintained body weight for 135 days" on a diet containing cottonseed flour as the sole source of minerals.

In their second article Richardson and Green (*12*) have attempted to repeat some of our work with extracts of cottonseed. Instead of using unheated cottonseed kernels, as we did, they used kernels heated to 120° C. Thus, they fail to find toxic the ether extract of petroleum-

ether-extracted kernels. Their results may be due to the influence of previously heating the kernels to 120° C., thus possibly decomposing some of the gossypol, and to incomplete extraction so that the remainder was left in the three fractions, the extracts and the residue. They also assume that the ethyl-ether extract of petroleum-extracted kernels is always 2 per cent of the weight of the kernels. This is the case only with long-continued extractions.

SUMMARY

Raw cottonseed kernels contain about 0.6 per cent of gossypol and are highly toxic to rats. Ether extraction renders the material nontoxic and gives a highly toxic extract containing about 2 per cent of gossypol. Gossypol fed in milk diets in amounts equivalent to those contained in the raw cottonseed diets has proved as toxic as raw cottonseed. Gossypol may be quantitatively removed from the ether extract by precipitation as its insoluble anilin compound. The extract is thus rendered nontoxic to rats. The insoluble anilin compound of gossypol is not toxic because of its insolubility. Gossypol prepared from this compound possesses its original toxic properties.

Cottonseed meal is much less toxic than raw cottonseed, owing mainly to the oxidation of gossypol during cooking.

Cottonseed meal, ether-extracted cottonseed, and gossypol have been fed to small pigs in pens under comparable conditions. Cottonseed meal has been found definitely injurious, while the ether-extracted raw seed does not appear to cause cottonseed-meal poisoning. Gossypol has been found toxic to pigs.

If the presence of an injurious substance in the meal is disregarded, a diet of cottonseed meal and corn meal has nutritive limitations which may, under restricted conditions of living, lead to failure of pigs to thrive. Such failure is a phenomenon distinct from cottonseed-meal poisoning.

Outdoor exercise, access to forage and soil, and improved diets tend to postpone or avert cottonseed-meal poisoning of swine. The deficiency hypothesis that cottonseed-meal poisoning of swine is similar to beriberi is untenable.

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PLATE 1

Effect of feeding cottonseed feeds to pigs:

A.—Pig 3, showing condition on the ninety-fourth day on a feed containing ether-extracted cottonseed kernels.

B.—Pig 3, showing condition on the fiftieth day.

C.—Pig 4, showing condition on the twenty-seventh day on a feed containing vitamines.

D.—Pig 1, showing condition on the fiftieth day on a feed containing cottonseed meal. See figure G.

E.—Pig 2, showing condition on the twenty-seventh day on a feed containing gossypol.

F.—Pig 3, showing condition on the twenty-seventh day on a feed containing ether-extracted cottonseed kernels.

G.—Pig 1, showing condition on the fiftieth day on a feed containing cottonseed meal.

FRUIT-FLY PARASITISM IN HAWAII DURING 1916

By C. E. PEMBERTON, *Assistant Entomologist*, and H. F. WILLARD, *Fruit-Fly Quarantine Inspector, Mediterranean Fruit-Fly Investigations, Bureau of Entomology, United States Department of Agriculture*

Since the introduction of parasites of the Mediterranean fruit fly (*Ceratitis capitata* Wiedemann) into the Territory of Hawaii in 1913, by the Territorial Board of Agriculture and Forestry, more or less continuous notes have been kept, from month to month, indicating the extent of parasitism exerted upon the larvæ of the fruit fly by these parasites and by other species subsequently brought in. Papers presenting summaries of these records, separately for the years 1914 [1] and 1915,[2] have already been published. Opportunity for special investigations of fruit-fly parasites in Hawaii in 1916 has made possible the accumulation of much careful data on fruit-fly parasitism during this year, of much the same nature as that given in the publications just cited, and it is the purpose of this paper to give, possibly more in detail, some results of the work in 1916.

It is felt that a separate record of the conditions of parasitism as existing in Hawaii in 1916, three years after the first and probably the most important of these introductions, will be of no little value and interest to entomologists, by way of comparison with the parasitism in 1914 and 1915, for interesting developments in connection with the question of general parasite introductions and as a necessary contribution to the history of fruit-fly parasitism in Hawaii.

The tabulation during most of the year of the exact degree of infestation of large quantities of host fruits of the fruit fly, from many localities, has been an important part of this work (Tables I–III). All fruits collected for parasitism records on their contained maggots have been accurately counted and placed in separate boxes over sand. The fruit is then kept in the boxes until practically all the contained fruit-fly larvæ have developed, emerged, and entered the sand below for pupation. A record of the total number of larvæ thus developing and pupating is secured. The larvæ quickly pupate after leaving the fruit. The pupæ are placed in vials and later carefully counted in determining the degree to which they have been parasitized. The filing of exact data of this nature from year to year is necessarily the most reliable and positive method of ascertaining the actual degree to which the parasites now

[1] BACK, E. A., and PEMBERTON, C. E. PARASITISM AMONG THE LARVÆ OF THE MEDITERRANEAN FRUIT FLY (C. CAPITATA) IN HAWAII IN 1914. *In* Bien. Rpt. Bd. Comrs. Agr. and Forestry Hawaii, 1913/14, p. 153–161. 1915.

[2] ——— ——— PARASITISM AMONG THE LARVÆ OF THE MEDITERRANEAN FRUIT FLY (C. CAPITATA) IN HAWAII IN 1915. *In* Jour. Econ. Ent., v. 9, no. 3, p. 306–311. 1916.

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established are contributing toward a control of the fruit fly. Such data not only indicate the extent of fruit infestation from various localities, but also the amount of parasitism among the larvæ from month to month and the seasonal efficiency of each parasite.

Seasonal differences in the value and prolificness of certain species of the introduced parasites have been most striking. This is suggested by an examination of any of the parasite notes from almost any locality and by a comparison of emergences of different species for each month of the year. But most convincing proof that seasonal differences exist is obtained by the inspection of records from fruit collected from the same localities month by month. Some species of trees in Hawaii bear fruit, normally a host of the fruit fly, almost continuously throughout the year. The systematic collection of fruit from such trees and the filing of exact data bearing on the extent of parasitism of fruit-fly larvæ secured from such individual trees throughout the year have thrown most light upon the seasonal values of the different parasites.

Mention of possible fluctuations in the abundance of different species has already been made.[1] The work of 1916, wherein it has been possible to concentrate parasites in fruit collections from individual trees, has most impressively shown the rise of the parasite *Diachasma tryoni* Cameron in the summer and fall of the year and its certain decline during the winter and particularly the spring months. Changes in temperature, of no great magnitude, alone seem responsible for this. The parasite *Opius humilis* Silvestri, more hardy and prolific than any of the other introduced species, has been overshadowed by the other species, particularly by *D. tryoni*, and has had its seasonal rise and fall directly the reverse and entirely dependent upon the rise and fall of this species of Diachasma. The slight seasonal changes have little visible effect upon the activities of *Opius humilis*, however, for in the winter and spring, with the decrease in abundance of *D. tryoni*, it rapidly ascends and becomes the most effective check upon the fruit fly (Table III). These interrelations are treated elsewhere by the writers.

The problem of control of the fruit fly in Hawaii through parasites is only partially solved. The four species already established are accomplishing a certain control, particularly in the coffee districts, but a casual survey of the extent of infestation of most host fruits as shown in Table I will convince one of the continued destructiveness of this pest in Hawaii. An average parasitism of 40 per cent of all of the larvæ developing is, numerically considered, of much importance; but from the standpoint of the practical needs of the horticulturist it brings little relief.

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TABLE I.—*Extent of infestation of host fruits by larvæ of Ceratitis capatata in Hawaii during 1916*

Host fruit.	Number of fruits collected.	Number of larvæ of *C. capitata* emerging.	Average number of larvæ per fruit.
Kamani (*Terminalia catappa*)	15, 723	149, 415	9. 5
Mango (*Mangifera indica*)	1, 317	2, 291	1. 7
Coffee (*Coffea arabica*)	41, 605	21, 224	. 5
Strawberry guava (*Psidium cattleianum*)	13, 825	22, 017	1. 6
Black myrobalan (*Terminalia chebula*)	6, 615	46, 639	7. 0
Peach (*Prunus persica*)	669	13, 738	20. 5
Rose-apple (*Eugenia jambos*)	1, 258	7, 001	5. 5
Chrysophyllum monopyrenum	1, 956	4, 034	2. 0
Brazilian plum (*Eugenia braziliensis*)	4, 398	3, 808	. 8
French cherry (*Eugenia uniflora*)	7, 627	6, 617	. 8
Mimusops elengi	11, 883	63, 017	5. 3
Ochrosia elliptica	77	240	3. 1
Kamani (*Calophyllum inophyllum*)	218	737	3. 3
Bestill (*Thevetia neriifolia*)	1, 532	5, 540	3. 6
Averrhoa carambola	159	214	1. 3
Chinese orange (*Citrus japonica*)	1, 588	4, 843	3. 1
Noronhia emarginata	5, 296	9, 032	1. 7
Guava (*Psidium guajava*)	1, 791	12, 248	6. 8

Table I shows few fruits that have a yearly average infestation of less than two larvæ per fruit. Considering the large quantity of fruits collected during the year, from which the records have been made, an average of two larvæ per fruit is high. It means that great numbers of fruits from all localities are nearly always heavily infested. The fruits collected for such a record are not selected with the purpose of obtaining only heavily infested fruits or only sound fruits. All available fruits that have matured are gathered and brought in whether infested or not. In this manner the exact average condition of fruit-fly abundance, injury, and parasitism is obtained.

To refer again to the tables particular attention should be called to the mango (*Mangifera indica*), guava (*Psidium guajava*), *Mimusops elengi*, *Noronhia emarginata*, and the Chinese orange (*Citrus japonica*). Great numbers of fruit-fly larvæ develop in these fruits and are but slightly parasitized, as shown in the total column for these fruits in Table II. Certain characters of these fruits prevent the parasites from reaching the larvæ within. This in part accounts for the constant presence of this pest, in spite of the establishment of parasites well adapted to the conditions of the country and of great prolificness.

TABLE II.—*Percentage of larval parasitism of Ceratitis capitata in Hawaii, 1916*[a]

Host fruit.	Month of collection.	Number of larvæ emerging during first 2 to 6 days.	Percentage of parasitism.				
			Opius humilis.	*Diachasma tryoni.*	*Diachasma fullawayi.*	*Tetrastichus giffardianus.*	Total.
Kamani (*Terminalia catappa*)	January	115	22.6	......	......	......	22.6
Do	March	2,792	30.5	0.6	......	0.5	31.6
Do	April	9,558	46.1	1.1	0.1	.03	47.33
Do	May	1,391	24.7	13.3	.07	......	38.07
Do	June	3,094	6.8	16.5	.4	......	23.7
Do	July	3,569	2.4	7.3	.9	.1	10.7
Do	August	4,017	8.4	27.7	......	.9	37.0
Do	September	3,526	11.8	53.9	......	.2	65.9
Do	October	3,403	15.5	58.5	.4	1.7	76.1
Do	November	2,299	8.3	51.5	......	2.3	62.1
Do	December	1,408	9.0	49.0	......	1.2	59.2
Mango	June	283	9.5	3.8	6.7	.3	20.3
Do	July	299	9.7	12.0	3.3	1.3	26.3
Do	August	47	6.3	4.2	......	2.1	12.6
Do	September	53	1.8	5.6	......	......	7.4
Do	October	39	5.1	12.8	......	......	17.9
Coffee *b*	February	390	60.7	6.4	......	......	67.1
Do	March	62	4.8	......	66.1	......	70.9
Do	April	1,621	60.3	29.7	4.3	......	94.3
Do	May	105	57.1	5.7	.9	.9	64.6
Do	June	131	85.4	......	......	......	85.4
Do	August	308	17.2	52.9	9.4	......	79.5
Do	October	288	59.0	10.4	12.1	......	81.5
Do	November	1,192	22.3	11.3	27.9	......	61.5
Do	December	643	12.2	14.6	17.1	......	43.9
Strawberry guava	April	2,705	38.1	.1	2.7	.1	41.0
Do	May	48	27.1	......	......	......	27.1
Do	June	979	10.2	6.7	6.0	......	22.9
Do	July	1,013	10.3	46.0	3.5	.4	60.2
Do	October	189	3.1	14.8	......	.5	18.4
Do	November	34	8.8	41.1	5.8	......	55.7
Black myrobalan	October	3,081	11.2	3.8	.2	.3	15.5
Do	November	2,518	8.5	8.6	1.0	1.1	19.2
Do	December	1,319	27.4	5.3	1.8	12.8	47.3
Peach	March	2,311	3.2	......	.08	......	3.28
Do	April	951	15.0	.5	.4	......	15.9
Rose-apple	May	170	4.1	37.6	2.3	......	44.0
Do	June	1,089	2.2	62.4	.1	......	64.7
Do	July	14	......	57.1	......	......	57.1
Chrysophyllum monopyrenum	January	996	6.5	1.1	1.5	......	9.1
Do	February	702	1.2	......	.5	......	1.7
Do	March	378	1.0	......	1.0	......	2.0
Do	April	78	74.3	......	12.8	......	87.1
Do	May	634	57.0	......	.9	......	57.9
Brazilian plum	June	1,306	19.5	16.6	22.3	......	58.4
Do	November	78	7.6	16.7	29.4	......	53.7
Do	December	33	6.0	3.0	21.2	......	30.2
French cherry	January	41	34.1	......	14.6	......	48.7
Do	March	95	8.4	......	......	......	8.4
Do	April	25	64.0	......	......	......	64.0
Do	May	114	10.5	32.4	9.6	......	52.5

a Most of the fruits represented in this table were collected about Honolulu at low elevations; the coffee, however, was collected on the island of Hawaii, in addition to localities in Honolulu, and much of it came from points 1,000 to 2,000 feet above sea level.

b The June collection of coffee came from the Waianae Mountains, where only *Opius humilis* was established.

TABLE II.—*Percentage of larval parasitism of Ceratitis capitata in Hawaii, 1917*—Con.

Host fruit.	Month of collection.	Number of larvæ emerging during first 2 to 6 days.	Percentage of parasitism.				
			Opius humilis.	*Diachasma tryoni.*	*Diachasma fullawayi.*	*Tetrastichus giffardianus.*	Total.
French cherry	June	479	4.8	6.2	11.4	......	22.4
Do	July	60	1.6	1.6	48.3	......	51.5
Do	December	862	3.4	10.7	8.4	......	22.5
Mimusops elengi	January	181	4.4	......	.5	......	4.9
Do	February	314	.6	......	......	......	.6
Do	March	976	3.5	......	......	......	3.5
Do	April	10,514	8.4	......	.01	.02	8.43
Do	May	2,535	10.0	.3	.3	.1	10.7
Ochrosia elliptica	May	103	15.5	36.8	23.3	......	75.6
Do	June	18	16.6	5.5	22.2	......	44.3
Kamani (*Calophyllum inophyllum*)	January	819	.8	......	.2	.2	1.2
Do	March	210	1.9	......	......	......	1.9
Do	April	167	4.7	......	......	......	4.7
Do	December	116	.8	......	......	......	.8
Bestill	March	18	5.5	......	......	16.6	22.1
Do	May	22	......	......	......	......	.0
Do	June	85	7.0	9.4	9.4	......	25.8
Do	August	173	10.9	3.4	2.3	1.7	18.3
Do	September	274	3.6	2.5	22.6	5.8	34.5
Do	October	392	3.3	3.3	19.1	9.6	35.3
Do	November	84	......	......	39.2	7.1	46.3
Do	December	159	1.2	......	16.9	13.8	31.9
Averrhoa carambola	September	32	40.6	......	......	......	40.6
Do	October	84	13.0	......	......	......	13.0
Chinese orange	January	143	4.1	......	.7	......	4.8
Do	March	319	......	......	.2	......	.2
Do	July	39	......	7.7	5.1	......	12.8
Do	August	258	3.8	1.5	2.7	......	8.0
Do	September	76	23.6	......	......	......	23.6
Noronhia emarginata	June	1,767	.2	1.5	.05	......	1.75
Do	July	853	.4	1.6	......	......	2.0
Guava	May	403	3.4	.4	......	......	3.8
Do	June	781	2.0	.2	......	......	2.2
Do	July	248	2.5	3.6	.4	......	6.5
Do	September	1,670	5.2	2.1	.05	......	7.35
Do	October	496	4.6	......	......	......	4.6

The data in Tables I and II covering the guava are of unusual interest. This shrub grows wild and luxuriantly over most of the uncultivated portions of the islands up to an elevation of 1,500 to 2,000 feet, and fruits throughout the year. It is not generally considered by the layman of Hawaii as a favored host of the fruit fly, though of the 18 host fruits given in Table I, it stands fourth in degree of infestation, showing, from 1,791 fruits collected during the year, an average infestation of 6.8 larvæ per fruit. Infestation of this fruit is not easily detected until it has decayed. The larvæ are small and nearly all inconspicuous at the time the fruits are picked and eaten or converted into preserves or jelly. This fruit, though heavily infested in most localities, protects the larvæ

from parasite attack and thus constantly liberates great numbers of flies throughout the year and serves but in a small measure toward the building up of favorable quantities of parasites.

TABLE III.—*Total parasitism by month of all larvæ of Ceratitis capitata collected in Hawaii during 1916*

Month.	Number of larvæ.	Percentage of parasitism.				
		Opius humilis.	*Diachasma tryoni.*	*Diachasma fullawayi.*	*Tetrastichus giffardianus.*	Total.
January	2,295	5.5	0.4	1.0	0.08	6.98
February	1,406	17.6	1.7	.2	....	19.5
March	7,161	13.7	.2	.6	.2	14.7
April	21,619	34.8	2.1	.7	.04	37.64
May	5,525	19.6	6.1	.9	.09	26.69
June	10,013	7.8	15.5	4.5	.009	27.809
July	6,134	3.7	13.0	1.8	.02	18.52
August	4,803	8.8	27.0	.8	.9	37.5
September	5,631	9.7	34.0	1.1	.4	45.2
October	7,972	13.8	27.4	1.7	1.4	44.3
November	6,205	11.0	25.2	6.7	1.4	44.3
December	4,540	13.3	20.9	5.3	4.6	44.1

A comparison of fruit-fly parasitism data secured during the years 1914, 1915, and 1916 would indicate that the parasites now present in the Territory have reached their maximum degree of development and can hardly be expected to attain a greater control of the fruit fly than that evidenced in 1916. There has been some variation during the past three years in the activities of the different species introduced, as already noted in regard to the fluctuations in abundance of *Diachasma tryoni* and *Opius humilis;* but the check upon the work of this pest by the present parasites can hardly exceed its present limits. Some hope, however, is yet felt for the parasite *Tetrastichus giffardianus* Silvestri. It has gradually increased in numbers about Honolulu since its establishment late in 1914. Certain valuable points in its favor may enable it, after further acclimatization and general adaptation to new environment, to exceed the work of the braconids and thus increase the total average parasitism.

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IRRIGATION EXPERIMENTS ON APPLE-SPOT DISEASES[1]

By CHARLES BROOKS, *Pathologist*, and D. F. FISHER, *Assistant Pathologist, Bureau of Plant Industry, United States Department of Agriculture*

THE PROBLEM

The present paper deals with the effects of soil-water supply upon bitter-pit, Jonathan-spot, and certain other nonparasitic spot diseases of the apple (*Malus sylvestris*). It also includes notes upon the relation of the time of picking to the development of apple-spots in storage.

BITTER-PIT

HISTORICAL REVIEW

Bitter-pit was first described by Wortmann (*22*)[2] under the name "*Stippen.*" It has been frequently discussed in the publications of the State Experiment Stations under the name "Baldwin-spot" and was referred to in the Nineteenth and Twentieth Reports of the New Hampshire Experiment Station as "fruitpit." Lewis (*8*) and Allen (*1*) apparently used the term "fruitpit" to refer to the troubles discussed later in this paper under the name "cork," and McAlpine (*9–12*) apparently included cork and also drouthspot under the name "bitter-pit."

Various explanations have been offered as to the cause and nature of bitter-pit. Wortmann (*22*) reported that the disease was due to abnormal transpiration conditions and that varieties of apples in which the water was conducted most readily from the deeply seated cells to replace that lost by transpiration were least susceptible.

Sorauer (*19, p. 80*) thought that the pits were produced by rupturing of the cells during the process of swelling. In a later publication (*20, p. 116-169*) he stated that the disease was worst on porous dry soils and suggested that the pits were produced by an overrapid maturing of certain cell groups resulting from the checking effect of drouth upon the accumulation of organic material.

Evans (*5*) reported that the disease was due to a bursting of cells in the apple tissue that resulted from the sudden checking of transpiration at night while the root action of the tree remained vigorous.

[1] Studies on Fruit Rots and Spots: III.

[2] Reference is made by number (italic) to "Literature cited," p. 136–137.

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Ewart (*6, 7*) concluded that the disease was the result of local poisoning and mentioned spray materials and the toxic salts of the soil as possible causes.

White (*21*) considered that the disease was the result of the poisoning effects of arsenical compounds and other spray materials.

McAlpine (*9–12*) thought that the disease was produced by a shortage of water in the affected tissue and that the condition might be brought about either by transpiration exceeding the water supply or by the growth of the pulp tissue being too rapid to allow time for the formation of the new vascular tips needed to supply it with water. He found that there was slightly less of the disease on trees receiving two irrigations than on those receiving one.

DESCRIPTION OF BITTER-PIT

Bitter-pit makes its first appearance as water-soaked bruiselike spots on the surface of the apple. The epidermal tissue is at first entirely normal, the spotted effect being due to the breaking down of cells in the subepidermal region. The spots soon become depressed into rather definite pits, 2 to 6 mm. in diameter, hemispherical in shape, and fairly regular in outline. They develop a higher color than the surrounding surface of the apple, becoming a deeper red than the adjacent tissue when occurring on the colored portion of the fruit and a darker green when on the lighter parts (Pl. 2, A). As the disease advances farther, the spots take on a brownish color owing to the dead pulp cells beneath the epidermal layers, and in late stages of the trouble the affected area may entirely lose its normal color, becoming a deep brown (Pl. 3, A). The diseased tissue is dry and spongy, the cells are collapsed but still full of starch, and the cell walls show no sign of thickening or disintegration. The affected tissue often has rather a bitter taste, and this together with the sunken nature of the spots has given rise to the term "bitter-pit."

The pits are usually associated with the terminal branches of the vascular bundles, and the surface spotting is often accompanied by a browning of the vascular tissue deeper in the fruit, giving the appearance of numerous brown spots in the flesh when the apple is cut (Pl. 2, B). This internal browning is especially common in the tissue within a centimeter of the surface of the apple. While the internal browning and surface pitting are commonly associated, either may occur without the other.

Bitter-pit is often confined to the calyx half of the apple. Baldwin, Northern Spy, Grimes, Jonathan, and Yellow Bellflower are especially susceptible to the disease; and Rome Beauty and Winesap are fairly resistant; but almost all varieties are sometimes affected.

Bitter-pit is very similar in appearance to rosy-aphis stigmonose, but the latter disease is not accompanied by a browning of the vasculars and the subepidermal tissue has a firmer texture and a darker color than is the case with bitter-pit. Stigmonose is found only on limbs that

were infested with aphids earlier in the year, and the spots usually appear several weeks before picking time, while bitter-pit is rather evenly distributed over the tree and is found only on mature or nearly mature fruit. Bitter-pit can be distinguished from fruitspot by the fact that with the latter disease there is an almost entire absence of subepidermal browning, and the spots have an irregular outline and a flecked or speckled appearance.

EXPERIMENTAL WORK

The writers were convinced by earlier investigations that bitter-pit was not due to fungi or bacteria. They had frequently seen unsprayed fruit that was seriously affected with the disease, thus making the theory that spray materials were responsible for the trouble seem entirely untenable. Drouth had frequently been mentioned as a cause of bitter-pit, and several writers had suggested an excessive or uneven water supply as a possible cause, but little experimental data had ever been furnished in support of any of these theories. The question of the influence of soil-water supply seemed to the writers to be an extremely important one, and a series of experiments were started to determine the effect of irrigation upon the disease.

The work has been located at Wenatchee, Wash. The climate of the section is arid, but little precipitation occurring from April till October, thus making the trees almost entirely dependent upon irrigation for their soil-water supply during the growing season. Except where otherwise mentioned, the water was applied by the furrow method (Pl. 4, A). The contrasts in the amount of water on the different plats were secured by varying the frequency and duration of the irrigations and, in some cases, by differences in the number of furrows supplying water to the row and by variations in the head of water at the flume.

The amount of water in the soil was determined by means of samples taken with a soil auger. In the beginning of the work samples were taken at depths of 6, 18, 30, 42, and 54 inches—that is, from the middle of each of the first 5 successive feet of soil—but in the final experiments, as reported later, samples were taken only from the one or two depths that seemed most important in determining the condition under which the tree roots were working in the particular orchard.

Some difficulty was found in securing samples that represented the average moisture conditions of the tree row. The lateral movement of soil water is very slow, resulting in considerable contrast between the amount of moisture beneath the irrigation furrow and a few feet from it, especially in the upper layers of the soil. Samples were usually taken at a distance from the furrow equal to one-fourth the space between the furrows, thus securing soil from a point midway between the wettest and dryest areas. The plan of sampling was always the same for the different plots of a particular orchard. Samples were taken at intervals of 7 to 10 days, and usually just before and 1 or 2 days after an irrigation, thus obtaining a record of the extremes in soil-water conditions.

As soon as obtained, the samples were transferred to tin cans with tightly fitting lids, and the cans immediately closed. The weight of the fresh soil sample was determined and a second weighing made after the soil had been reduced to constant weight in a drying oven, the difference between the two weighings being taken as the moisture content of the sample. The percentage of saturation was determined by comparing the moisture content of the sample with the total water-holding capacity of the soil. In the experiments of 1914 and 1915 the latter was secured by taking the average water-absorbing capacity of a large number of samples, but in 1916 saturation tests were made on each soil sample.

Notes were taken on the amount of bitter-pit at picking time, and later notes were taken to determine the increase in storage. The apples were cut open at the time of the last note-taking and a record made of the amount of internal browning. An apple was counted as affected with bitter-pit if it had either internal or external evidence of the disease, but very few apples showed internal browning that did not also have the external pitting.

EXPERIMENTS ON GANO APPLES IN 1914

The irrigation experiments were begun in 1913. The results of the first season were of little value, since the main trouble in the experimental orchards was found to be stigmonose instead of bitter-pit. In 1914, the data from the most promising orchard were lost on account of mistakes of the picking crew, but some interesting contrasts were obtained in an orchard of Gano apples. The trees in the latter orchard were 11 years old and thrifty; the soil was a volcanic ash, uniform in texture to a depth of 6 feet. The orchard had been under clean cultivation but at the time of the experiments was sown to vetch. There were four trees in each plat. The soil-moisture condition for the season is shown in figure 1. All of the plats became quite dry the middle of August on account of trouble with the irrigation canals.

TABLE I.—*Percentage of bitter-pit on Gano apples in 1914*

Plat No.	Irrigation treatment.	Total number of apples.	Percentage of apples of following sizes:					Percentage of bitter-pit.
			3¾ to 4 inches.	3½ to 3¾ inches.	3¼ to 3½ inches.	3 to 3¼ inches.	2¾ to 3 inches.	
1	Heavy throughout season	129	2.7	25.2	27.0	36.6	8.5	7.0
2	Medium throughout season	152	.8	18.8	30.6	41.9	7.9	2.6
3	Light throughout season	176	.0	7.0	30.6	39.9	22.5	2.3
4	Medium till Aug. 1, then light	144	.6	21.9	23.9	41.0	12.6	.0

The crop was quite heavy, averaging about 25 bushels per tree. There was no bitter-pit on the fruit at picking time. Five boxes of apples from each plat were placed in cold storage and held for three months. Tab e I gives the results of notes taken at the end of this storage period.

The results make it evident that heavy irrigation favored bitter-pit, and also increased the size of the apples. It can be seen that there is

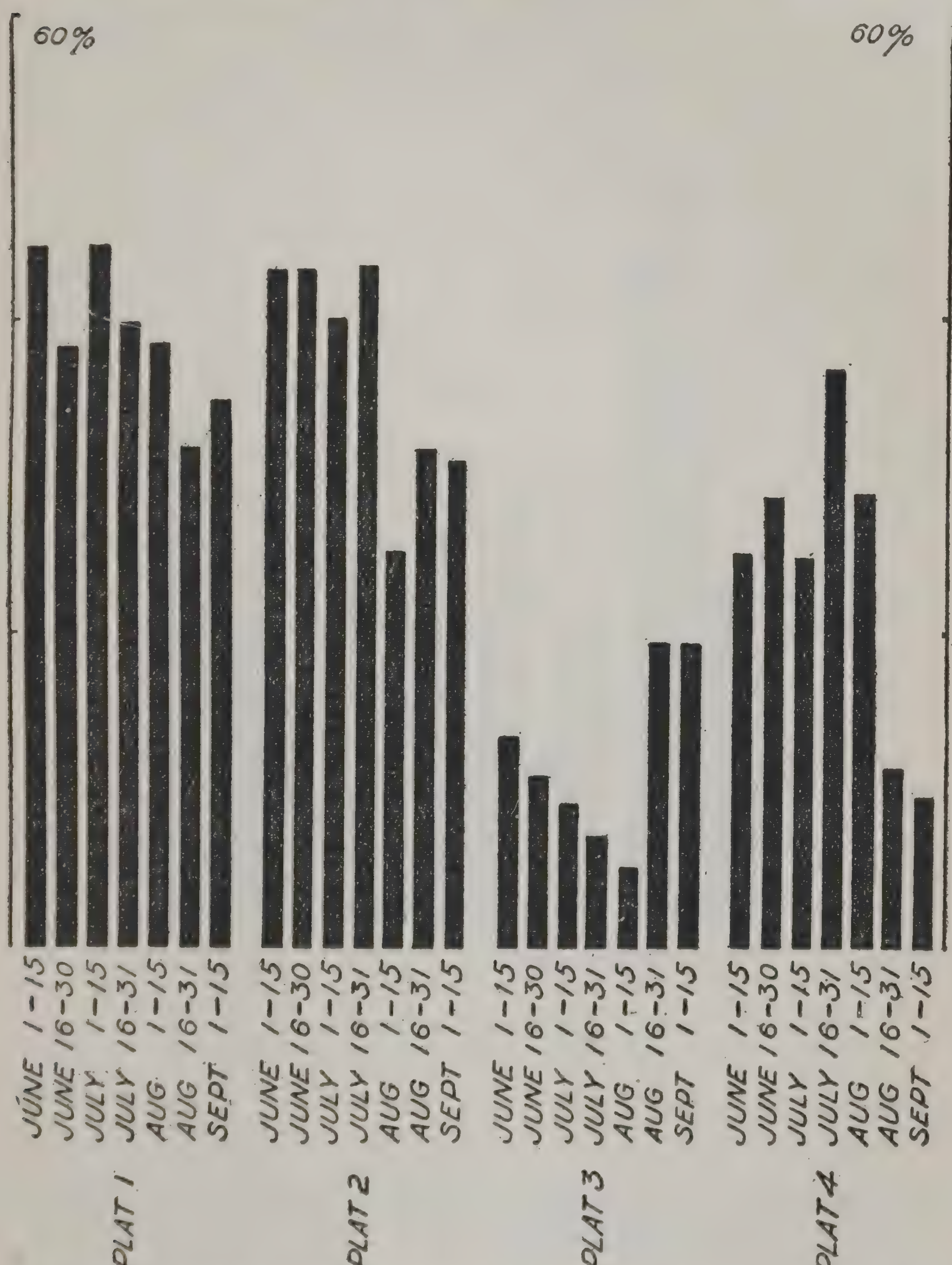

FIG. .—Diagram showing the soil-moisture conditions in irrigated plats of Gano apples in 1914. The results show the average percentage of saturation for each half month based on the average of soil samples at depths of 24 and 36 inches.

a very close relation between the size of the apple and the amount of the disease, but there is hardly sufficient parallelism to justify the conclusion that the increase in bitter-pit is entirely due to increase in size.

EXPERIMENTS ON GRIMES APPLES IN 1915

Similar irrigation experiments were carried out in 1915 on Grimes apples. The experimental plats were located on bottom land along the Wenatchee River. The soil was of an alluvial nature composed of

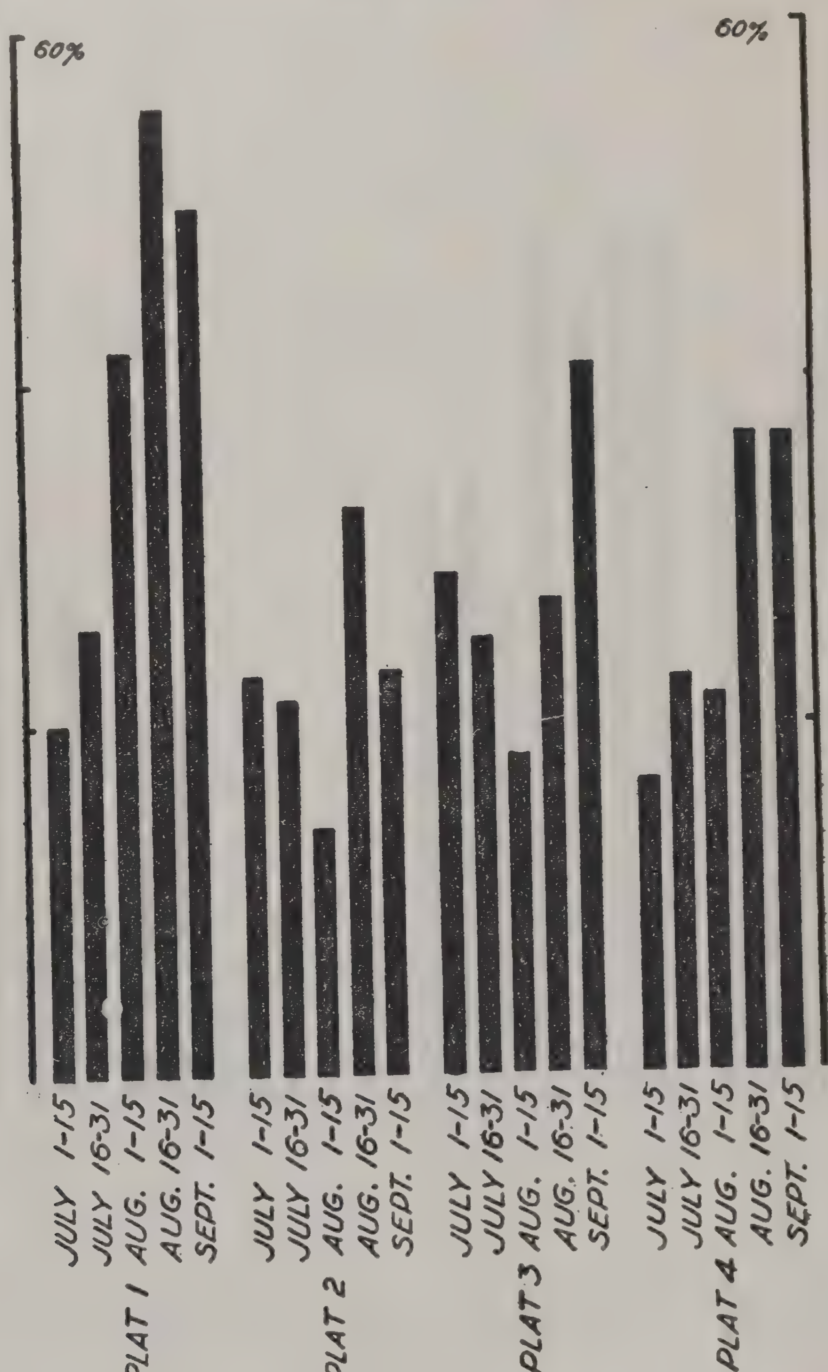

FIG. 2.—Diagram showing the soil-moisture conditions in irrigated plats of Grimes apples in 1915. The vertical bars show the average percentage of saturation for each half month based on the average of soil samples at depths of 24 and 36 inches.

medium heavy sandy loam with considerable clay and was uniform to a depth of 3 feet. It was kept under cultivation in the tree row. The trees were 5 years old and making a vigorous growth. They were quite uniform in size and vigor and satisfactory in every respect for comparative experiments. Three trees were used in each plat. The fruit was

kept practically free from stigmonose by means of a late dormant spray of lime-sulphur and nicotin sulphate.

The irrigation of the orchard was not satisfactory because of a shortage of water resulting from trouble with the canals. A cloudburst on July 26 gave an indiscriminate watering to all the plats. Irrigations were made according to plan on August 24 and September 12. The soil moisture conditions for the latter part of the season are shown in figure 2.

It will be noted that in spite of the unfavorable conditions a decided contrast in soil moisture was secured on the different plats. Plat 1 was given heavy irrigation throughout the season; plat 2, medium; plat 3, light; and plat 4, medium, followed by heavy. The percentages of soil saturation given do not indicate any decided contrast between plats 2 and 3, but the condition of the trees in the two plats made it very evident that a distinct contrast in soil-water conditions had been secured.

The yield in the orchard was light, being about a bushel to the tree. The fruit was gathered on September 22, about 10 days later than the average commercial picking of Grimes apples in that section. It was placed immediately in cellar storage at a temperature of about 50° F. Notes were taken on bitter-pit seven days later. The results are shown in Table II.

TABLE II.—*Percentage of bitter-pit on Grimes apples. September 29, 1915*

Plat No.	Irrigation treatment.	Total number of apples.	Percentage of apples affected with bitter-pit.		
			Windfalls.	Picked fruit.	Total.
1	Heavy	299	90	43	56
2	Medium	222	30	17	25
3	Light	156	36	14	23
4	Medium till Aug. 24, then heavy	175	77	49	59

The contrasts are quite striking and make it evident that heavy irrigation tended to increase the amount of bitter-pit. It is interesting to note that plat 4, which was heavily irrigated late in the season, showed a greater percentage of the disease on the picked fruit than plat 1, which was heavily irrigated early as well as late.

All of the fruit that was apparently free from bitter-pit was returned to cellar storage and notes were taken again on November 9. The results are given in Table III. All of the percentages but those in the last column are based on the number of apples returned to storage and not on the number in the original yield from the plats.

TABLE III.—*Percentage of bitter-pit on Grimes apples in storage. November 9, 1915*

Plot No.	Irrigation treatment.	Total number of apples.	Percentage of apples of the following sizes.		Percentage of apples that developed bitter-pit from Sept. 29 to Nov. 9.			Total percentage of bitter-pit developed by Nov. 9.
			Larger than 2⅝ inches.	2⅝ inches and smaller.	Apples larger than 2⅝ inches.	Apples 2⅝ inches and smaller.	Total.	
1	Heavy	132	81.8	18.2	63.9	4.2	53.0	79
2	Medium	166	92.2	7.8	48.4	.0	44.6	59
3	Light	106	83.0	17.0	33.7	11.0	29.2	52
4	Medium till Aug. 24, then heavy	72	98.6	1.4	63.4	.0	62.5	84

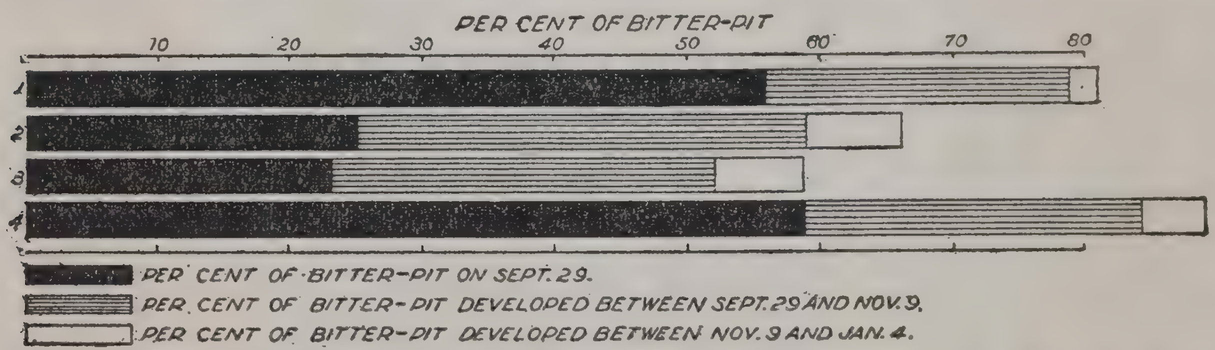

FIG. 3.—Diagram showing the amount of bitter-pit on Grimes apples in 1915. The black portions of the bars indicate the percentage of apples affected with bitter-pit one week after picking; the shaded portions, the amount developed between September 29 and November 9; and the white portion, the amount between November 9 and January 4. All of the percentages are based on the number of apples at the beginning of the experiment. See figure 2 for soil-moisture conditions.

A study of Table III shows that nearly all of the bitter-pit occurred on the apples that were larger than 2⅝ inches. The percentages in the next to the last column show that the contrasts in bitter-pit on the stored samples were similar to those found a week after picking, and indicate the importance of orchard conditions in determining the susceptibility of the fruit in storage. These percentages are estimated on the basis of the sound apples left on September 29. If the original number of apples were taken as a base in estimating percentages, these contrasts would partially disappear, as is shown in figure 3; but this would be an unfair comparison, so far as determining behavior in storage is concerned, as a large number of the apples had already been eliminated from the experiment. The last column in Table III shows the total amount of bitter-pit to November 9, estimated on the basis of the original number of apples.

The sound fruit from the above experiment was returned to cellar storage and a third set of notes taken on January 4, the fruit being cut open at this time to determine the amount of internal streaking or browning. But very few specimens of bitter-pit were found, and these gave but little contrast between the fruit from the different irrigation plats.

The results obtained on Grimes apples in 1915 are shown in graphic manner in figure 3. The contrasts for the season are similar to those obtained on September 29.

EXPERIMENTS ON GRIMES APPLES IN 1916

In 1916 the experiments were continued in the Grimes orchard described above. Five trees were included in each plat. It was possible

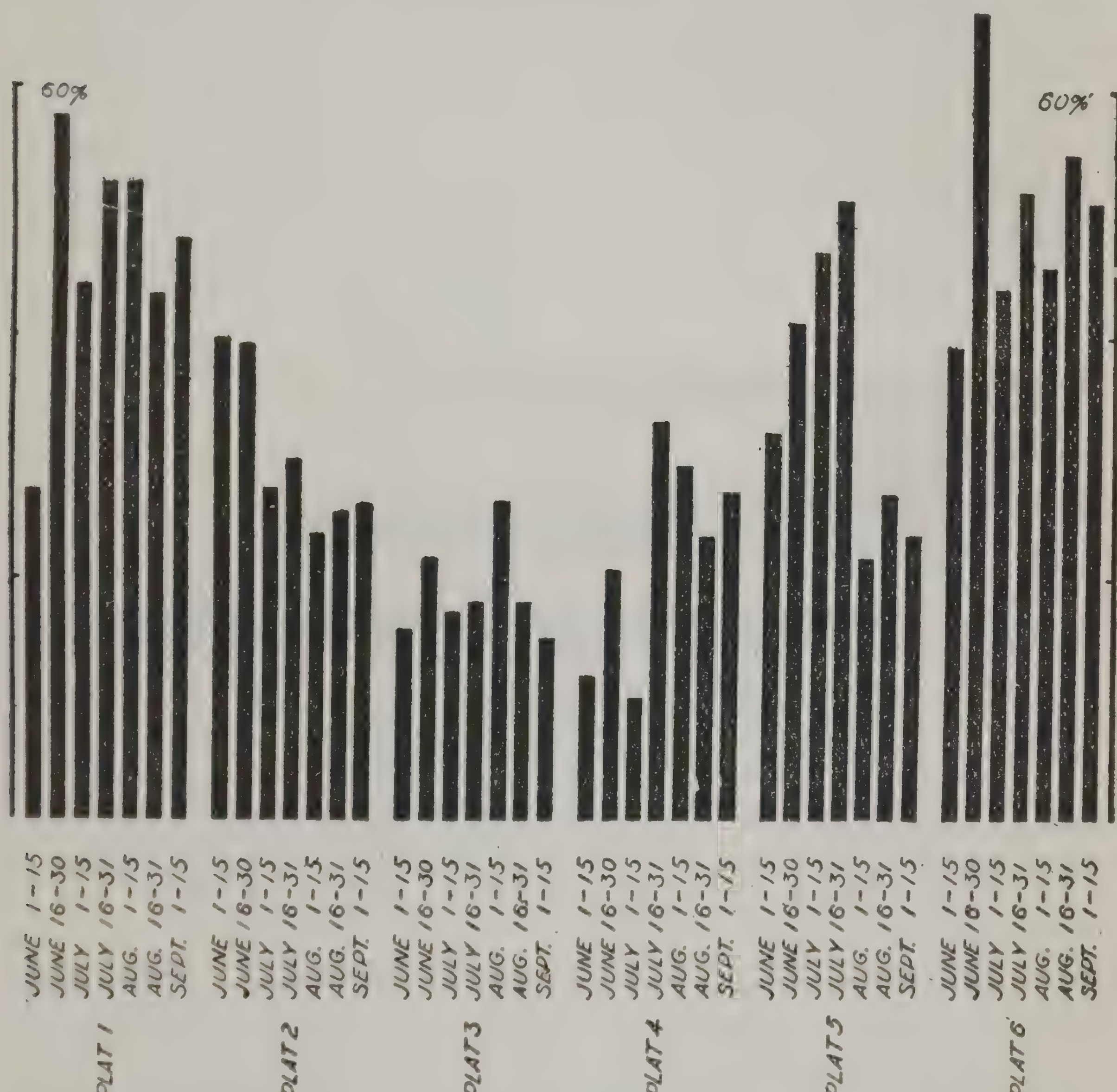

FIG. 4.—Diagram showing soil-moisture conditions in irrigated plats of Grimes apples in 1916. The results show the average percentage of saturation for each half month based on the average of soil samples at depths of 24 and 36 inches. Plat 1 received heavy irrigation throughout the season; plat 2, medium; plat 3, light; plat 4, medium till late in July, then heavy; plat 5, medium in June, heavy in July, and light in August and September; and plat 6, heavy throughout, with the exception of a sudden drop to medium in July.

to carry out the irrigation schedule much more satisfactorily than in the preceding year. The soil-moisture conditions for the season are shown in figure 4.

The fruit on plat 3 was noticeably smaller than that on the other plats as early as August 1, and by the close of the season the effects of irrigation were quite evident in the size of the fruit from the various plats. At picking time the fruit on plat 3 was found to be somewhat riper and more highly colored than that on the other plats. The apples were picked

on September 16. The crop was uniform and quite heavy, making it possible to secure approximately 2 bushels from each tree for storage.

The fruit was placed in cellar storage in open packages. Hygrothermograph records showed that from September 19 to October 18 the temperature of the cellar averaged 55° F., and the relative humidity approximately 55 per cent; that from October 18 to November 9 the average temperature was 48° F. and the average relative humidity 68 per cent, and that from November 9 to March 20 the temperature was fairly constant at 35° F., the relative humidity averaging 80 per cent. Notes were taken on September 19, when the fruit was picked, and on October 18, November 9, and March 20. At the time of the last note taking the apples were cut open and a record made of the internal browning as well as the bitter-pit spots. The vascular bundles of about half the pitted apples were browned, but the apples that showed no external

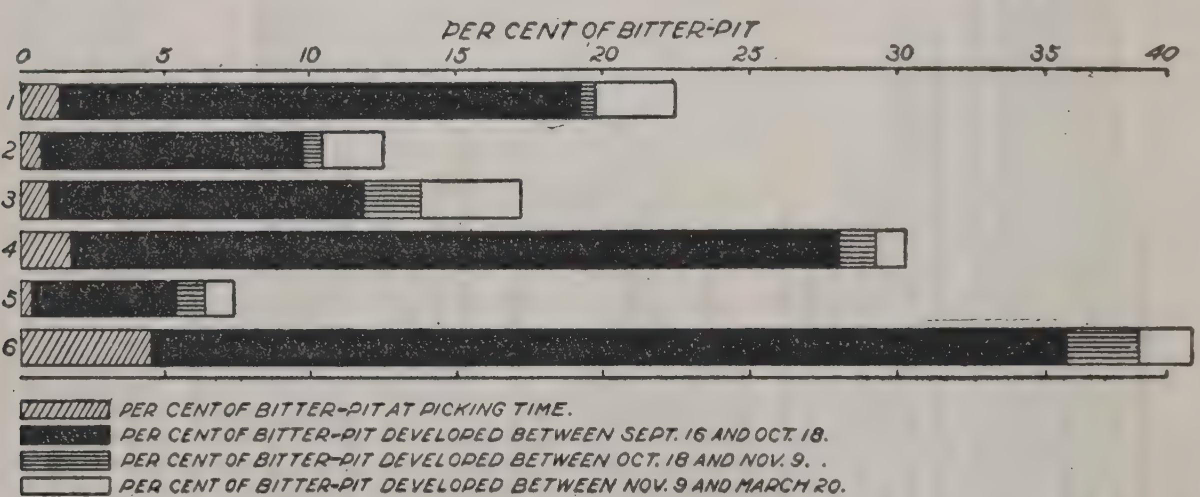

FIG. 5.—Diagram showing the amount of bitter-pit on Grimes apples in 1916. The diagonally shaded portions of the bars indicate the percentage of apples having bitter-pit at picking time; the solid portions, the percentage developed between September 19 and October 18; the horizontally shaded portions, the amount developed between October 18 and November 9; the white portions, the amount developed between November 9 and March 20. See figure 4 for soil-moisture conditions.

evidence of bitter-pit were free from internal browning. The bitter-pit results are given in Table IV. The percentages in the first and last columns are based on the total number of apples, those in the other columns on the number of sound apples at the previous note taking.

TABLE IV.—*Percentage of bitter-pit on Grimes apples*

Plat No.	Percentage of apples affected with bitter-pit.				
	At picking time, Sept. 19.	Developed between Sept. 19 and Oct. 18.	Developed between Oct. 18 and Nov. 9.	Developed between Nov. 9 and Mar. 20.	Total.
1	1.5	19.8	0.8	3.3	23.0
2	1.0	9.8	1.0	2.0	12.7
3	.7	12.1	2.0	4.1	17.6
4	1.3	28.7	1.5	2.0	31.1
5	.4	5.3	1.6	.6	7.6
6	4.1	35.2	5.5	3.2	40.8

A study of the table shows that nearly all of the disease developed during the first month of storage. The contrast between the plats, however, makes it evident that the development of the disease was largely determined by orchard conditions. The apples from the heavily irrigated plats were in all cases more susceptible to bitter-pit than those from the lightly irrigated ones. The amount of disease was much greater on plats 4 and 6, which were irrigated heavily only late in the season, than on plat 1, which was heavily irrigated throughout the season. It was less on plat 5, which had heavy irrigation followed by light, than it was on plat 3, which received light irrigation throughout the season, or

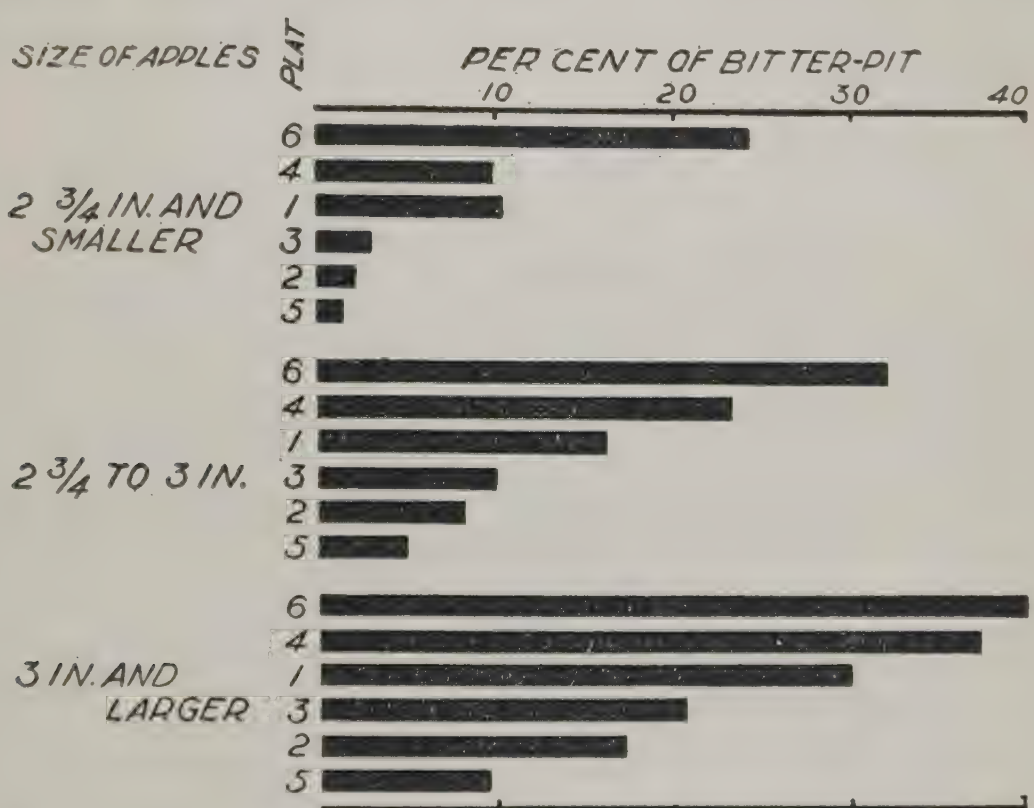

FIG. 6.—Diagram showing the relation of the amount of bitter-pit to the size of apples. The bars show the amount of disease on the different plats and are grouped according to size of apples. It will be noted that heavy irrigation increased the disease as much on the small fruit as on the large. For the irrigation of the different plats see figure 4.

on plat 2, which received medium irrigation throughout the season. The results indicate that the character of the irrigation during the last weeks in which the apples are on the trees largely determines the amount of bitter-pit developed in storage.

The total amount of bitter-pit for the season is shown in graphic manner in figure 5. All of the percentages are based on the original number of apples.

In the note taking of October 18 the apples were graded according to size, and the record on bitter-pit made accordingly. The results are given in Table V and figure 6.

TABLE V.—*Percentage, according to size, of Grimes apples affected with bitter-pit. October 18, 1916*

Plat No.	Total number of apples.	Percentage of apples of various sizes.					Percentage of apples of various sizes affected with bitter-pit.					Total percentage of bitter-pit.
		3½ to 3¾ inches.	3¼ to 3½ inches.	3 to 3¼ inches.	2¾ to 3 inches.	2¾ inches and smaller.	3½ to 3¾ inches.	3¼ to 3½ inches.	3 to 3¼ inches.	2¾ to 3 inches.	2¾ inches and smaller.	
1	776	0.4	10.8	30.8	37.9	20.1	100.0	52.4	20.9	13.6	10.9	19.8
2	775	.....	6.4	26.5	50.2	16.9	....	36.0	13.6	6.9	2.3	9.8
3	879	.1	10.3	28.3	48.8	12.5	100.0	14.5	26.7	9.6	3.6	12.1
4	560	.....	12.5	38.6	39.1	9.8	....	54.3	31.0	23.3	9.1	28.7
5	690	.....	6.7	24.8	45.1	23.4	....	7.0	15.2	5.1	1.2	5.3
6	715	.4	20.7	31.2	40.8	6.9	100.0	31.9	50.0	31.5	24.5	35.2

The large apples were much more susceptible to bitter-pit than the small ones, but evidently size can not be taken as a measure of susceptibility, since the small apples on the heavily irrigated plats often developed more disease than the large ones on the lightly irrigated ones. (Table V; fig. 6.) A study of the table shows, however, that the same soil conditions that favored bitter-pit also tended to increase the size of the fruit, the plats standing in practically the same order as to percentage of apples larger than 3¼ inches as they do in percentage of bitter-pit.

EXPERIMENTS ON JONATHAN APPLES IN 1915

Irrigation experiments were made on Jonathan apples similar to those already reported on Grimes. The work was carried out in an orchard at Wenatchee, Wash. The soil was a rich gravelly loam, with a considerable percentage of clay, underlain at a depth of 16 inches with a layer of medium fine gravel. For several years previous to the beginning of the experiments the orchard had been heavily manured with slaughterhouse refuse, and during the time of the experiments it was kept in alfalfa. The trees were 6 years old, and there were 5 trees in each plat. The experiments were begun in 1915. Breaks in the irrigation canals at various times and a rainstorm on July 26 made it impossible to secure much contrast in the different plats before the first of August. All the trees but those of plat 1 were extremely dry the latter part of June and the first half of July. A further report of this condition is given later in this paper under the head "Drouthspot." There was a shortage of water several times in August, plat 5 suffering severely from drouth at this time and finally losing more than 75 per cent of its foliage and considerable of its fruit (Pl. 4, B). Plat 3 suffered from drouth the latter part of August, but no defoliation occurred. Plat 2 was practically as wet as plat 1 during the latter part of July and first of August, but became quite dry about the middle of August. The moisture conditions for the season are given in figure 7.

The first picking was made on September 3, when the apples were rather green, a second on September 17, when they were right for commercial picking; and a third, October 1 when the fruit was dead ripe. In most cases a bushel of apples was secured from each tree at each picking. There was practically no bitter-pit on the fruit at picking time. The apples were placed in cellar storage at an average temperature of about 47° F., and notes were taken November 10. The results are given in Table VI.

All apples more than 2⅝ inches in diameter were counted as large, and the others as small. There was little contrast as to size in the fruit

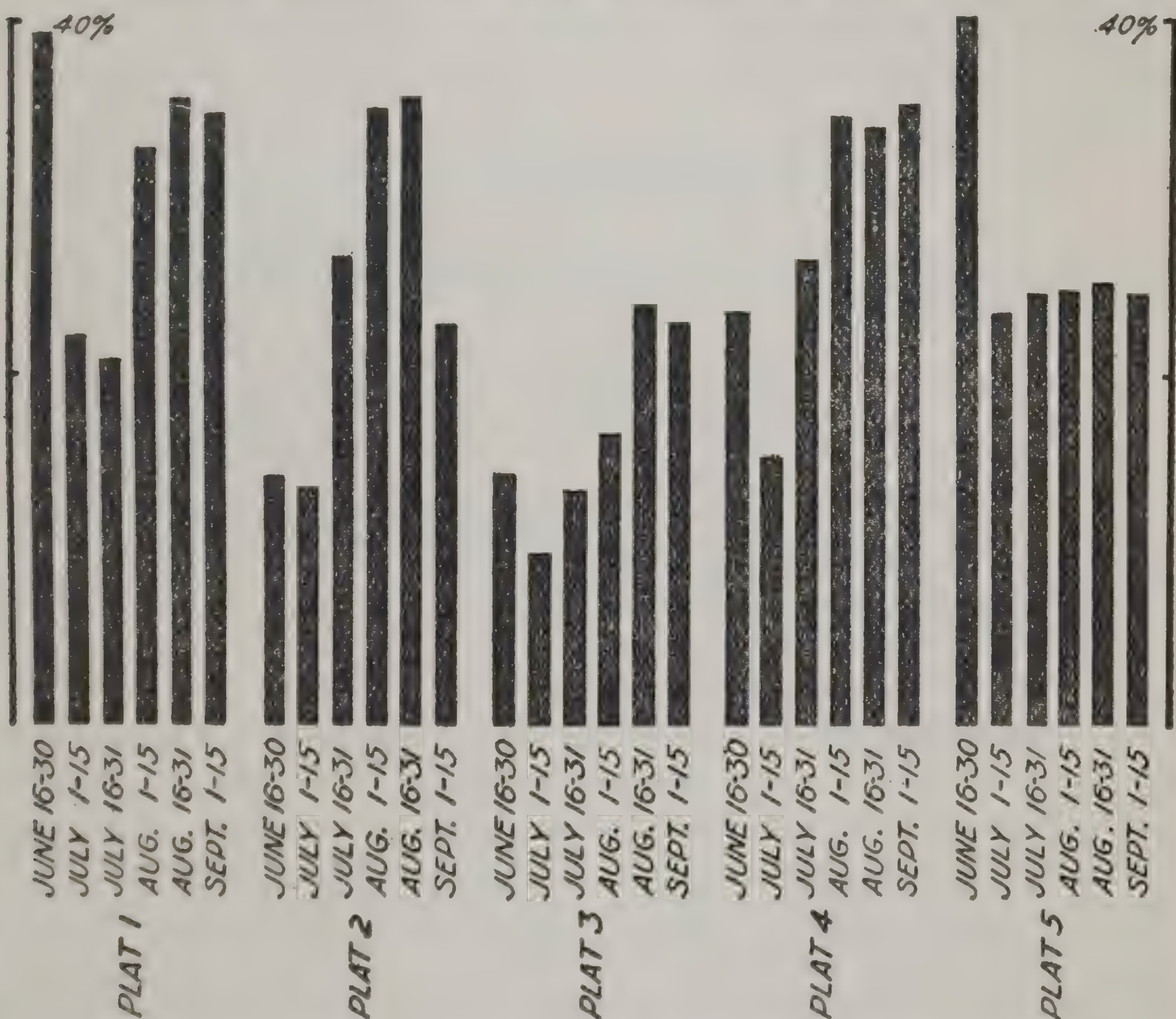

FIG. 7.—Diagram showing the soil-moisture conditions in irrigated plats of Jonathan apples in 1915. The average percentage of saturation is given for each half month and is based on soil samples taken at a depth of 16 inches. Plat 1 was to receive heavy irrigation throughout the season; plat 2, medium; plat 3, light; plat 4, medium in August, then heavy; and plat 5, heavy till August 1, then light. The schedule was followed as closely as the water supply would allow.

of the different pickings, and all three were combined to obtain the data given on size.

The large apples again have much more bitter-pit than the small ones. The apples of the first picking had more than twice as much bitter-pit as those of the second and those of the second several times more than those of the third. It might be suggested that a part of this contrast should be attributed to the fact that the earlier pickings had been in storage longer, but the later development of the disease in storage gives no support for this hypothesis. The more mature fruit was apparently much less sus-

ceptible to the disease. A study of the total bitter-pit as given in the last column of Table VI shows effects from irrigation similar to those obtained on Grimes apples. The fruit from the trees receiving medium irrigation followed by heavy irrigation late in the season had the most bitter-pit, and that from the trees irrigated heavy both early and late the next in amount. As has already been mentioned, the contrast between plats 1 and 2 in the amount of irrigation was not as great as intended; the latter, however, received less water and had less pit than the former. Plats 2 and 5 had but little bitter-pit, even on the large apples. The fruit from plat 2, however, was of an inferior quality on account of the sunscald that resulted from the defoliation of the trees.

TABLE VI.—*Percentage of Jonathan apples affected with bitter-pit. November 10, 1915*

Plat No.	Irrigation treatment.	Percentage of apples of following sizes.		Percentage of apples affected with bitter-pit.								
				First picking.		Second picking.		Third picking.		Total.		
		Larger than 2⅝ inches	2⅝ inches or smaller.	Apples larger than 2⅝ inches	Apples 2⅝ inches or smaller.	Apples larger than 2⅝ inches	Apples 2⅝ inches or smaller.	Apples larger than 2⅝ inches	Apples 2⅝ inches or smaller.	Apples larger than 2⅝ inches	Apples 2⅝ inches or smaller.	Large and small
1	Heavy	91.9	8.1	32.9	22.7	15.0	16.7	4.0	22.2	18.5	22.0	18.6
2	Medium	82.4	17.6	32.5	5.9	11.7	2.0	1.3	.0	16.1	2.3	13.6
3	Light	66.9	33.1	13.7	8.2	5.4	1.4	.0	.0	5.9	3.8	5.7
4	Medium, followed by heavy	92.9	7.1	44.4	16.7	23.0	8.3	1.9	.0	25.8	9.1	24.6
5	Heavy, followed by severe drouth	49.8	50.2	4.5	4.4	.0	.0	......	......	3.4	3.3	3.4

The above fruit was held in cellar storage and a second examination made on February 7. At this time the apples were cut open, and any that had either browning of the vascular tissue or surface pitting were counted as affected with bitter-pit. The results are given in Table VII, the percentages being computed on the number of apples that were free from bitter-pit at the time of the last note-taking. There was little contrast in the amounts of disease on the different pickings, and the three are considered together.

TABLE VII.—*Percentage of bitter-pit on Jonathan apples. February 7, 1916*

Plat No.	Irrigation treatment.	Percentage of apples that developed bitter-pit in storage from November 10 to February 7.			Total percentage for season.
		Large Apples.	Small Apples.	Large and small apples.	
1	Heavy	7.6	5.5	7.4	24.6
2	Medium	2.1	.6	1.3	14.7
3	Light	3.6	1.9	3.3	8.8
4	Medium, then heavy	1.9	.0	1.6	25.8
5	Heavy, then very light	.9	1.2	1.0	4.3

But little bitter-pit had developed on any of the apples during the three months of cellar storage. This may have been because the susceptible apples had already been eliminated, or may have been due to the fact that the apples were in an open package and finally became slightly shriveled. The relative amounts of disease on the apples from the various irrigation plats is little different from that given in Table I. The results for the season are shown in the last column of Table VII and also in figure 8.

EXPERIMENTS ON JONATHAN APPLES IN 1916

The bitter-pit experiments were continued in 1916 in the Jonathan orchard already described. The irrigation conditions were much more

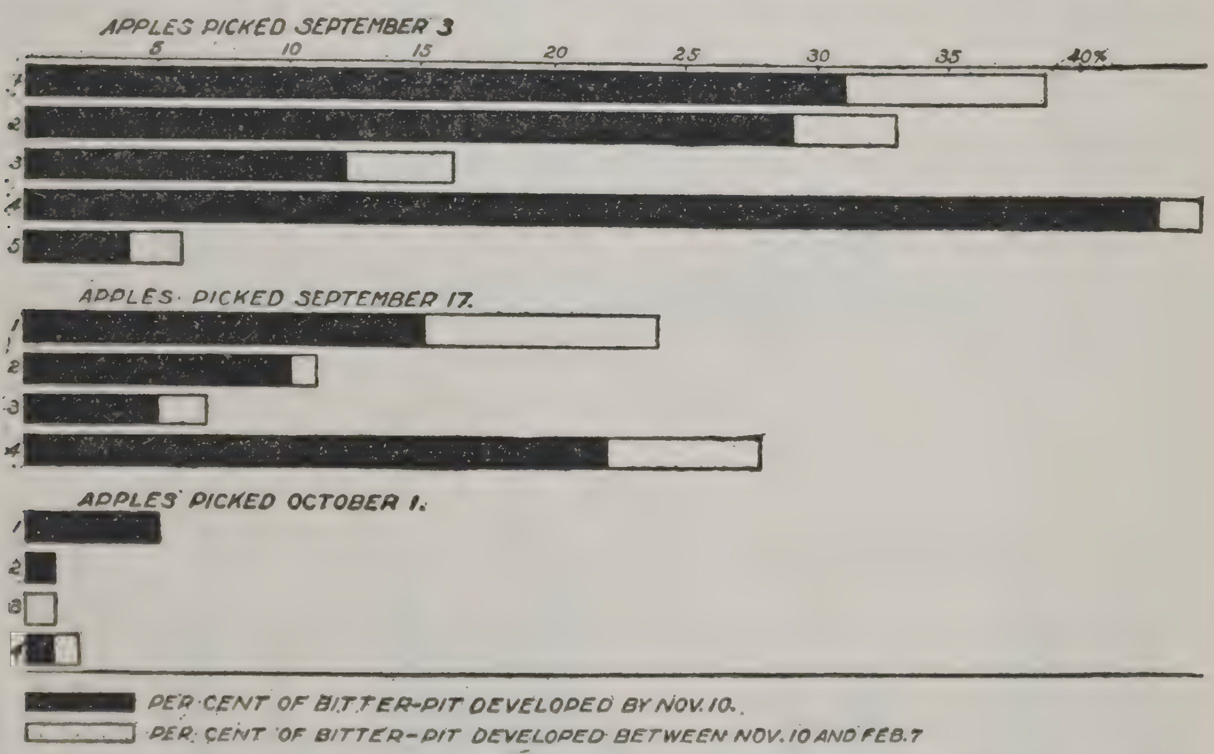

Fig. 8.—Diagram showing the amount of bitter-pit on Jonathan apples in 1915. The solid portions of the bars indicate the percentage of apples affected with bitter-pit on November 10, the white portions the percentage developed between November 10 and February 7. All of the percentages are based on the number of apples at the beginning of the experiment. See figure 7 for soil-moisture conditions.

satisfactory than in 1915. The percentages of soil saturation maintained on the different plats are shown in figure 9.

All of the trees were in vigorous condition except those of plat 3, which were apparently suffering from the effects of the drouth of 1915. The apples of this plat were very highly colored, while those of plats 5 and 7 were rather low in color. The first picking was made on September 22 and a second on October 2. The apples of the first picking were undercolored and immature, while those of the second were well colored and suited for commercial picking. Approximately 3 bushels of apples were saved from each plat in the first picking, and approximately 2 bushels from each in the second, and placed in cellar storage. There was no bitter-pit on the apples at picking time and none had developed by October 24. The results obtained from notes taken on November 14

and on March 18 are given in Table VIII. From the time of the storage of the fruit till November 14 the average temperature of the cellar was approximately 50° F., and the average relative humidity about 61 per cent. From November 14 to March 18 the temperature averaged 38° F., and the relative humidity 80 per cent.

TABLE VIII.—*Percentage of Jonathan apples affected with bitter-pit in 1916*

Plat No.	Irrigation treatment.	Percentage of apples affected with bitter-pit.					
		Nov. 14.			Mar. 18.		
		First picking.	Second picking.	Total.	First picking.	Second picking.	Total for year.
1	Heavy	3.0	0.0	1.5	4.9	2.3	3.5
2	Medium	2.6	.7	1.7	2.9	1.7	2.3
3	Light	1.5	.0	1.3	2.6	.0	2.2
4	Medium, followed by heavy	9.4	.0	5.6	11.1	2.2	7.5
5	Heavy, followed by light	.5	.4	.4	1.8	.3	1.4
6	Alternating, heavy, medium, heavy	1.3	.4	1.0	3.0	5.2	3.7
7	Alternating, heavy, medium, heavy, medium	2.9	.0	1.6	5.8	2.2	4.2

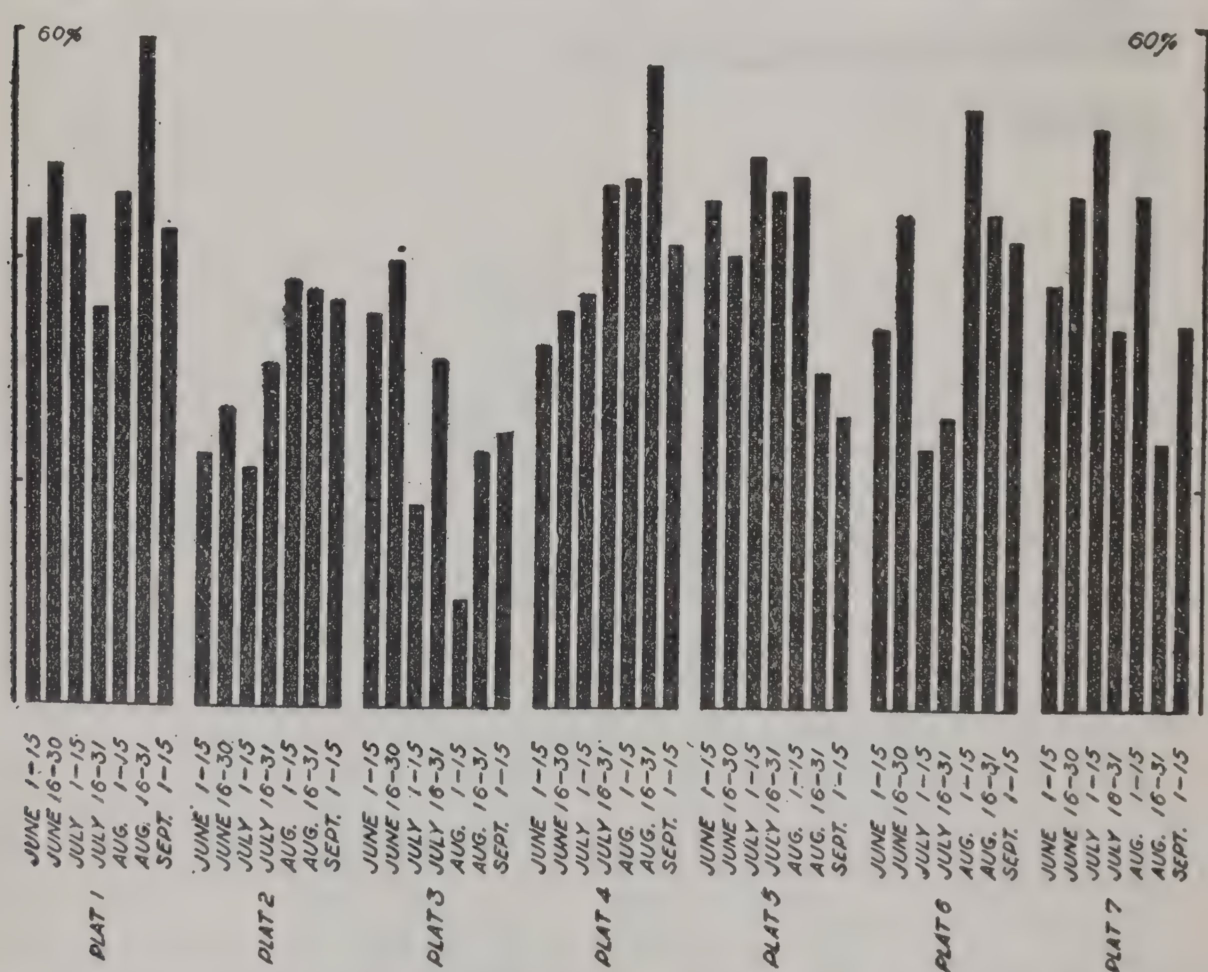

FIG. 9.—Diagram showing the soil-moisture conditions on plats of Jonathan apples in 1916. The average percentage of saturation is given for each half month and is based on soil samples taken at a depth of 16 inches. Plat 1 received heavy irrigation throughout the season; plat 2, medium; plat 3, light; plat 4, medium followed by heavy; plat 5, heavy followed by light; plat 6, heavy in June, medium in July, and heavy in August and September; plat 7, heavy till the middle of July, medium till August, heavy the first half of August, and medium the remainder of the season.

The relative susceptibility to bitter-pit of the apples from the different plats was the same as in previous experiments, the fruit from the trees receiving heavy irrigation late in the season having the largest amount of disease, that from those heavily irrigated throughout the season the next, and that from those receiving heavy irrigation followed by light having the least (fig. 10). As was found in the experiments of 1915, the apples from the early picking showed much greater susceptibility to bitter-pit than those of the late picking.

The size of the apples from the various plats and the relative susceptibility of the different sizes to bitter-pit is shown in Table IX.

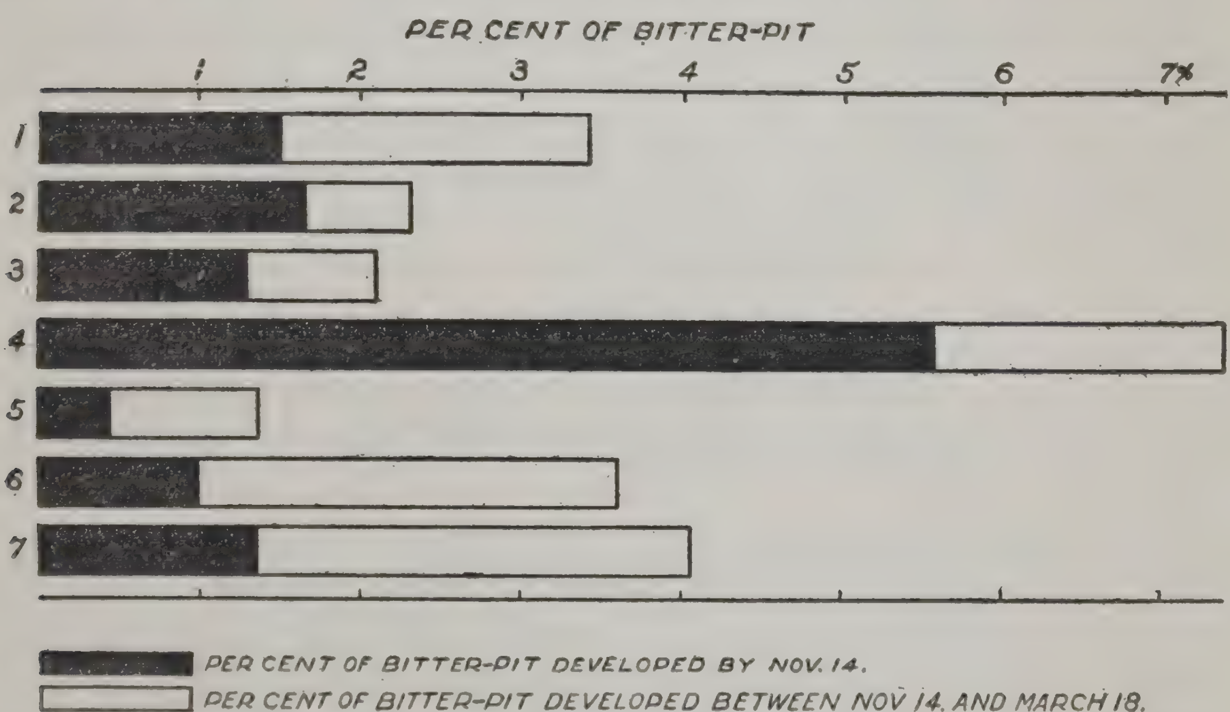

Fig. 10.—Diagram showing the amount of bitter-pit on Jonathan apples in 1916. The black portions of the bars indicate the percentage of apples affected with bitter-pit on November 14 and the white portions the percentage developed between November 14 and March 18. See figure 9 for soil-moisture conditions.

Table IX.—*Percentage, according to size, of Jonathan apples affected with bitter-pit. March 18, 1916*

Plat.	Total number of apples.	Percentage of apples of various sizes.					Percentage of apples of various sizes affected with bitter-pit.					Total percentage of bitter-pit.
		3½ to 3¾ inches.	3¼ to 3½ inches.	3 to 3¼ inches.	2¾ to 3 inches.	2¾ inches and smaller.	3½ to 3¾ inches.	3¼ to 3½ inches.	3 to 3¼ inches.	2¾ to 3 inches.	2¾ inches and smaller.	
1....	579	......	5.7	30.9	59.2	4.2	......	18.2	5.0	1.3	0.0	3.5
2....	593	......	.8	22.5	65.9	10.8	......	.0	3.8	2.2	1.5	2.3
3....	445	......	.7	1.1	40.5	57.7	......	.0	20.0	4.4	.4	2.2
4....	440	0.5	17.7	42.8	34.5	4.5	50.0	23.1	6.4	1.3	.0	7.5
5....	946	......	1.3	16.3	68.4	14.0	......	8.3	3.9	.9	.0	1.4
6....	566	.4	9.6	43.3	43.3	3.4	.0	20.0	2.9	1.2	.0	3.7
7....	542	......	4.1	32.4	59.6	3.9	......	9.1	6.3	3.1	.0	4.2

The plats receiving heavy irrigation late in the season had more large apples than the others. The amount of bitter-pit on the fruit of

a particular size was hardly sufficient to form a basis for conclusions, but it is evident that the disease was worse on the large apples than on the small, and that with the exception of one or two cases where there were but few apples heavy irrigation increased the amount of disease on the medium-sized as well as on the large fruit.

DISCUSSION OF RESULTS OF BITTER-PIT EXPERIMENTS

The results of the various experiments have been uniformly consistent in showing that heavy irrigation favors the development of bitter-pit. Heavy irrigation throughout the season has given less of the disease than medium irrigation followed by heavy, and light irrigation throughout the season has resulted in more bitter-pit than heavy irrigation followed by light. Heavy irrigation the first half of the season caused the trees to develop a more luxuriant foliage and probably produced a lower concentration of cell sap in the apples, both of which facts would tend to make the fruit less susceptible to the forcing effects of late irrigation. The amount of irrigation in August and September has apparently largely determined the amount of disease.

Sudden changes in the amount of soil water do not appear to have had any effect upon the amount of disease. No evidence has been found that bitter-pit is brought about by a rupture or bursting of the cells.

Large apples have been more susceptible to bitter-pit than small ones, but the increase in the disease from heavy irrigation has been almost as great on the small and medium sized fruit as on the large. This fact is brought out in Tables I, V, VI, and IX, and in a particularly striking manner in figure 6. Apparently apples are not susceptible to bitter-pit merely because they are large, but rather because of conditions that may sometimes accompany an increased growth.

The results as a whole point to the harmful effects of heavy late irrigation regardless of the size of the fruit. In looking for the final cause of the disease not only the direct growth-forcing effects of the water should be considered but also the effects of the excess water upon the soil flora and soil solutes. This subject will be more fully discussed in a later publication upon the effects of fertilizers.

JONATHAN-SPOT

HISTORICAL REVIEW

Jonathan-spot was first reported by Scott (*17*). He suggested the possibility that the trouble might be due to the effects of arsenate of lead. Later Scott and Roberts (*18*) gave a fuller report on the disease, showing that it could not be due to the effects of spraying and that while fungi were sometimes present in the spots they could not be taken

as the causal agency. They considered the disease of a physiological nature and found that it could be partially prevented by early picking, prompt cold storage, and early consumption after removal from storage.

Norton (*15*) reported that spots practically identical in appearance with the Jonathan-spot could be produced by the gases of ammonia and folmaldehyde.

Cook and Martin (*3*) considered Jonathan-spot to be a form of rot caused by a species of Alternaria. In a later report (*4*) they made a distinction between the small, nearly black, typical Jonathan-spots that were more commonly confined to the dark area of the skin, and the larger light-brown "Alternaria" spots that were more common on the lightest area of the skin. They reported that they were able to reduce the amount of the disease by keeping the apples covered with glassine bags during the latter part of the summer, and considered that this fact furnished further evidence that the spots were of fungus origin.

DESCRIPTION OF JONATHAN-SPOT

"Jonathan-spot" is the term applied to superficial black or brown spots that are especially common on Jonathan apples. The trouble is also found on Esopus, Yellow Newtown, Stayman Winesap, and other varieties. In the early stages of the disease only the surface color-bearing cells are involved and the spots are seldom more than 2 mm. in diameter, but later the spots may enlarge to a diameter of 3 to 5 mm., become slightly sunken and spread down into the tissue of the apple to a considerable depth. In this later stage of the disease rot fungi are often present, Alternaria being particularly common.

EXPERIMENTAL WORK

The Jonathan-spot experiments were carried out in the same Jonathan orchard and on the same apples as the bitter-pit experiments, and the details in regard to soil, irrigation, time of picking, and condition of storage have already been given.

In 1915, plat 5 suffered severely from drouth the latter part of the season, the trees finally losing more than three-fourths of their foliage and the fruit becoming badly bronzed by the sun. Plats 2 and 3 also became very dry in August but there was no defoliation. The soil moisture conditions for the season are given in figure 7. The first picking was made September 3. The fruit at this time lacked fully 10 days of being at its best stage of maturity for picking. A second picking was made on September 17 and a third picking on October 1. The fruit of the last picking was highly colored and dead ripe.

There was no Jonathan-spot at picking time. The results of notes taken on November 10 and February 1 are given in Table X.

TABLE X.—*Percentage of Jonathan apples affected with Jonathan-spot in 1915*

Date of picking.	Plat No.	Total number of apples.	Percentage of apples of following sizes.		Percentage of apples affected with Jonathan-spot.					
					November 10.			February 1.		
			Larger than 2⅝ inches.	2⅝ inches and smaller.	Apples larger than 2⅝ inches.	Apples 2⅝ inches and smaller.	Total.	Apples larger than 2⅝ inches.	Apples 2⅝ inches and smaller.	Total.
Sept. 3	1	281	84	16	24	11	22	52	50	51
	2	237	86	34	18	0	15	45	32	41
	3	338	62	38	5	3	4	54	72	62
	4	250	93	7	13	6	12	38	28	38
	5	179	49	51	2	3	3	22	18	20
Sept. 17	1	260	98	2	34	0	33	67	17	65
	2	291	81	19	18	4	15	48	45	47
	3	354	79	21	23	16	21	77	65	76
	4	195	94	6	17	8	16	93	67	92
	5	56	52	48	3	0	2	38	15	27
Oct. 1	1	183	95	5	17	22	18	79	33	77
	2	198	78	22	3	1	2	72	30	63
	3	267	60	40	11	4	8	79	35	61
	4	173	92	8	4	0	2	62	79	64
	5	......	......	......	......	......	......	......	......	......
Total for all pickings	1	724	92	8	26	12	25	64	47	62
	2	726	82	18	14	2	12	52	37	49
	3	959	67	33	14	6	11	71	58	67
	4	618	93	7	12	5	11	62	54	62
	5	235	50	50	3	3	3	26	18	22

There was more Jonathan-spot on the large apples than on the small ones, and at the time of the first note-taking there was more on the fruit of the first and second pickings than on that of the third. Irrigation apparently had but little effect upon the disease. The apples from plat 5 had the least Jonathan-spot; but, as already mentioned, these were badly sunburned and therefore not suitable for use in comparison with those of the other plats.

In 1916 the experiments were continued in the same orchard. All the trees were in a healthy, vigorous condition except those of plat 3. These were the same as used in plat 5 the preceding season and showed the effects of the previous year's drouth in their thin foliage and short twig growth. The soil-moisture conditions for the various plats are given in figure 9. Pickings were made on September 22 and October 2. The fruit from plats 5 and 7 was rather poorly colored, while that from plat 3 was very highly colored. The conditions of storage have already been given in connection with the notes on bitter-pit. The results for the season are shown in Tables XI and XII.

TABLE XI.—*Percentage of Jonathan apples affected with Jonathan-spot in 1916*

Plat No.	Irrigation treatment.	Percentage of apples affected with Jonathan-spot.					
		November 14.			March 18.		
		First picking.	Second picking.	Total.	First picking.	Second picking.	Total for year.
1	Heavy	1.3	0.3	0.8	55.8	82.8	65.4
2	Medium	3.8	0.0	2.1	51.2	57.3	54.2
3	Light	13.5	0.0	11.8	72.2	31.1	66.5
4	Medium, followed by heavy	7.9	.6	3.0	66.3	73.2	69.1
5	Heavy, followed by light	.5	0.0	.3	18.2	37.7	24.0
6	Alternating, heavy, medium, heavy.	3.4	.4	2.1	53.3	77.4	64.7
7	Alternating, heavy, medium, heavy, medium	.3	.6	.4	16.2	33.9	23.8

TABLE XII.—*Percentage, according to size, of Jonathan apples affected with Jonathan-spot in 1916*

Plat No.	Total number of apples.	Percentage of apples of various sizes.					Percentage of apples of various sizes affected with Jonathan-spot.					
		3½ to 3¼ inches.	3¼ to 3½ inches.	3 to 3¼ inches.	2¾ to 3 inches.	2¾ inches and smaller.	3½ to 3¾ inches.	3¼ to 3½ inches.	3 to 3¼ inches.	2¾ to 3 inches.	2¾ inches and smaller.	Total.
1	579	.....	5.7	30.9	59.2	4.2	......	72.8	61.5	66.8	66.6	65.4
2	593	.....	.8	22.5	65.9	10.8	......	60.0	53.4	52.9	62.5	54.2
3	445	.....	.7	1.1	40.5	57.7	......	......	60.0	58.3	73.2	66.5
4	440	0.5	17.7	42.8	34.5	4.5	......	55.0	68.7	77.7	70.0	69.1
5	946	.....	1.3	16.3	68.4	14.0	......	33.3	18.8	24.7	26.6	24.0
6	566	.4	9.6	43.3	43.3	3.4	100.0	38.2	65.3	69.8	63.2	64.7
7	542	.....	4.1	32.4	59.6	3.9	......	13.6	22.7	25.1	23.4	23.8

On November 14 the Jonathan-spot was worse on the apples of the first picking than on those of the second, but by March 18 this condition had in most cases been reversed. There was little contrast between the amount of disease on the fruit of different sizes. The contrasts between the irrigation plats were not very consistent, but in general indicated that heavy irrigation favored the disease. Plats 5 and 7, on which the fruit was lowest in color, had least of the disease.

DISCUSSION OF RESULTS ON JONATHAN-SPOT

The experiments on Jonathan-spot have furnished little in the way of consistent positive results. In both 1915 and 1916 the apples of the early picking had more of the disease than those of the late picking. In 1915 the large apples developed more Jonathan-spot than the small ones, but this did not hold in 1916. The results of both years gave some evidence that heavy irrigation was more favorable to the disease than light irrigation, but there was nothing to indicate that the amount of soil moisture was an important factor in determining the amount of Jonathan-spot.

OTHER PHYSIOLOGICAL SPOT DISEASES OF THE APPLE

DROUTHSPOT

The term "drouthspot" (*2*) has been applied to certain fairly large areas of dead brown tissue usually occurring near the surface of the apple, but sometimes found deeper in the flesh. The disease may appear at almost any stage in the development of the apple, but the fruit appears to be more susceptible after it is one-third grown. The spots are usually located on the blossom half of the fruit. In typical cases the trouble first appears as large, irregular, water-soaked spots that often have a reddish margin and are usually covered with drops of a yellowish, sticky ooze that is sweetish to the taste, and later hardens into a brittle, crystalline-like deposit (Pl. 3, D). At this stage the spots resemble fireblight infection (caused by *Bacillus amylovorus*) and are sometimes mistaken for it. Upon cutting the apple open a very shallow layer of dead brown tissue is found in the region of the vascular network just beneath the skin. Occasionally brown streaks follow the vascular bundles deeper into the apple pulp. The affected tissue is very bitter to the taste. The skin of the apple over the diseased area finally regains its normal appearance; but growth is arrested at this point, and the enlargement of the surrounding tissue soon gives rise to a much misshapen apple (Pl. 3, E, F). On account of its manner of development, the disease has sometimes been referred to as "spot-necrosis" (*13*). Mix (*14*) has given a full discussion of the characters of the disease as it occurred in the Champlain Valley of New York.

The above description applies particularly to the trouble as it has been observed on Winesap and Stayman Winesap apples in the irrigated sections of the West. It was first produced experimentally at Wenatchee, Wash., in 1913, by subjecting Winesap trees to a sudden and severe drouth when the fruit was about 1 inch in diameter. At about the same time it was observed at Peshastin, Wash., on Ben Davis trees that had suffered from a similar drouth. It occurred again at Wenatchee in 1914 and in 1915, always on trees that had been subjected to a sudden and severe drouth and that had been making a normal or vigorous growth earlier in the season. The drouth periods resulted from trouble with the irrigation canals. The affected trees were usually located on shallow soils or on soils underlain with coarse gravel at a slight depth, thus making them peculiarly susceptible to drouth.

In 1915 a series of drouth periods occurred, the first and most severe coming the latter part of June and the first of July, the second the latter part of July, and the third about the middle of August. At the time of the first drouth even the trees on deep soil began to suffer, and those on shallow soil lost a large percentage of both their foliage and fruit. The fruit that remained on the trees was much shrunken in size, sometimes being reduced to two-thirds its normal diameter. White Pearmain ap-

ples became very badly shriveled and wrinkled (Pl. 5, C), and Jonathan and Delicious apples showed slightly less serious effects; but with the return of irrigation water all of these regained their turgor without spotting. The Winesap and Stayman Winesap apples did not become as badly shriveled as the White Pearmain, but they developed typical drouthspots before they became shriveled. It was also observed that the oozing of the fruit sap, as well as the spotting of the fruit, preceded the renewal of irrigation. The apples subjected to the early drouth were also involved in the later ones, and the result was a series of spots on the same apples that could be distinguished as to time of formation by the color of the skin and the depth of the pitting.

On September 3 samples of fruit were obtained from the Jonathan trees that had suffered most severely from drouth, and on October 13 similar samples were secured from the Winesap, Stayman Winesap, and White Pearmain trees. All of the apples were placed in cellar storage until January 13 and were then cut open and examined. The Jonathan and White Pearmain apples had developed no spots, but their flavor was decidedly poor. With the Winesap and Stayman Winesap apples the spots had not enlarged, and there was but little brown tissue beneath the skin (Pl. 3, F). The flavor of the affected tissue was bitter and acrid, but that of the rest of the apple was normal.

The above trees that had suffered from drouth appeared to recover largely before the close of the season and their leaves came out normally the following spring; but a number of them died a few months later, and the remainder showed a lack of vigor throughout the summer. Their foliage was thin and they appeared to suffer from drouth even with a slight decrease in the percentage of soil moisture. The usual number of irrigations were made, and there were no real drouth periods; yet more than half of the apples on some of the Winesap and Stayman Winesap trees developed typical drouthspots. The weak condition of these trees and the death of others earlier in the summer probably resulted from the destruction of some of the smaller roots during the drouth of the preceding season.

CORK

The disease or group of diseases called "cork" may be similar to drouthspot in cause, but is distinctly different in many of its gross characteristics. Instead of being subepidermal, the spots are located in the pulp of the apple, often quite deeply seated and often closely associated with the larger vascular bundles (Pl. 5, B). The patches of dead, brown tissue are usually much larger than in the case of bitter-pit and much deeper than in drouthspot. They resemble the internal browning of the former disease, but are firmer in texture, more corky, and less spongy. Affected apples are often slightly less firm than others, and usually have a cheesy consistency when cut. When the spotting occurs near the core

only, there is usually no external marking to indicate the disease; but when the outer pulp tissue is affected, depressions occur over the dead spot, and the apple becomes more or less roughened or corrugated (Pl. 5, A, B). The development of the disease in the case of these corrugated apples is similar to that of drouthspot in many respects. It appears first as reddish stains on the surface of the apples, and these stained areas may gradually become water-soaked and covered with a sticky yellow ooze. Later the skin regains its normal color, but large areas of dead, brown tissue are left in the pulp.

Apples affected with cork are sometimes also affected with a condition known locally as "apple-blister." The trouble first appears as slightly raised brown or reddish spots on the skin of the apples (Pl. 5, E). The center of the raised portions is very hard and corky, but only the outer epidermal layers are involved. As the apple develops, the blisters crack and scale off, exposing a rough corky layer that has formed beneath. The later stages of blister have usually been found on apples that were also affected with cork, but blister appears early in the spring, very often becoming evident as soon as the petals have fallen.

Troubles identical with cork, or very similar to it, are quite widely distributed. They have been observed by the writers in the Wenatchee, Entiat, Spokane, Okanogan, and White Salmon districts of Washington, in the Willamette and Hood River Valleys of Oregon, in the Okanogan district of British Columbia, in the Champlain Valley of New York, and in various apple sections of Virginia and West Virginia. It is evident from McAlpine's reports (*9–12*) that the disease is of considerable importance in Australia.

McAlpine's (*9–12*) photographs indicate that he included the disease under the name "bitter-pit." Lewis (*8*) included "corerot" and "dryrot" as forms of fruitpit or bitter-pit. Allen (*1*) referred to the disease as "fruitpit." Mix (*14*) has very carefully distinguished between cork and bitter-pit. In British Columbia the disease is known as "malformation" and in Washington as "dryrot." A trouble known in Virginia as "York-spot," or "punky disease" (*16*), and in California as "hollow-apple" are apparently very closely related to cork.

The losses from the disease are usually local, but sometimes severe. At Entiat, Wash., in 1916, two carloads of apples from one 20-acre orchard were rendered worthless on account of cork. On the lower flats of the Okanogan Valley in British Columbia it is regarded as the most serious of all apple troubles, and in certain sections of the Hood River Valley, Oreg., it was the cause of considerable annual loss prior to the introduction of systematic irrigation.

The cause of cork is not known. Allen (*1*) has reported that fruitpit is worse on trees in a dry soil or in a soil lacking in organic matter. The disease is apparently not produced by fungi or insects. The writers have made repeated attempts to isolate an organism from the

affected tissue, but with negative results. Close observations have been made on the work of insects in orchards where the disease was serious, but no evidence has been secured to indicate the association of any insect with the production of the disease. Orchards affected with rosette are sometimes also affected with cork, but the latter disease occurs in orchards that are free from the former. In nearly every case where the disease has been observed either in the East or West, its occurrence in the orchard has been closely correlated with certain peculiar soil conditions; sometimes an excess of alkali or an outcropping of slate, but more often a shallowness or openness of the soil. In most sections cork has been most serious when there was a shortage in soil-water supply, either resulting from light rainfall or a lack of irrigation.

An orchard at Entiat, Wash., that has been seriously affected with cork has been under close observation for the past three years. The orchard is located on a low bench near the Columbia River, and has had a permanent cover crop of alfalfa. Soil samples from the orchard showed that in the sections where spotting had been most prevalent the surface soil was only about 3 inches deep and was composed of a coarse sand with only a small percentage of humus. The subsoil, which was more than 6 feet deep, differed from the surface soil only in the absence of the humus and was underlain with coarse gravel. In sections of the orchard where spotting had been less prevalent, the soil was found to be a much finer sand, and in sections where no spotting had occurred it was a typical volcanic ash, very fine in texture, closely compacted when wet, and very retentive of moisture. Soil-moisture determinations made soon after the spring rains showed that while the surface soils in the different orchard sections retained their moisture fairly well, the subsoil in the first section dried out quickly and that in the last section was very retentive of its moisture. It will be seen that the occurrence of the disease varied with the character of the soil, particularly with the water-holding capacity of the subsoil.

The irrigation of the orchard was inadequate. The furrows were 5 feet from the tree rows, and alfalfa growing near the trees and in the tree rows was yellow, frequently wilted, and very evidently suffering from lack of water. The trees suffered from drouth, especially in the spring, before the irrigations were begun. In 1916 the first irrigation was made several weeks later than usual and the trees became very dry. Later the apples developed an unusually high percentage of cork, the disease first appearing in blister form soon after the petals had fallen. The conditions in the orchard indicated that the soil-water supply was at least one important factor in determining the amount of disease.

The circumstances under which cork and drouthspot have occurred in the Champlain Valley have been quite fully described by Mix (*14*).

A special form of cork known in certain sections as "Yorkspot" and in others as "hollow-apple" has been found particularly common on

York Imperial apples and has also occurred on the Gano and the Esopus varieties. The disease has been under close observation for several years at Wenatchee, Wash., and in the summer of 1916 a careful study was made of it in orchards at Staunton, Va. In the latter case the disease was found only on York Imperial apples. It could not be correlated with any peculiar soil conditions, but was found decidedly worse on trees that were lightly loaded than on those with a medium or heavy crop. It was much worse on the south side of the tree than on the north side and slightly worse on the east side than on the west. It occurred almost exclusively on apples well exposed to sunlight, always on the blush side of the fruit, and always on fruit surfaces that would receive the oblique rather than the direct rays of light. The spots were similar in appearance to cork, but, instead of being scattered over the apple, were often located in a crescent-shaped line at the edge of the blush surface of the fruit. In some cases there was a definite ring almost entirely surrounding the point which received the most direct sunlight (Pl. 5, F, G). The skin of the apple was always normal, and the corky tissue beneath was usually indicated by surface depressions. While it seems probable that Yorkspot is in part an effect of drouth, its occurrence is undoubtedly greatly influenced by sunlight and possibly by soil conditions and other agencies.

The observations reported above seem to indicate that cork is a form of drouth injury; yet the disease appears to differ from typical drouthspot, both in characteristics and conditions of occurrences. With certain varieties of apples drouthspot can apparently be produced on any soil under conditions of sudden and extreme drouth. Cork seems to be the result of a less severe but more chronic drouth on trees located on certain peculiar soils, especially on soils that are lacking in humus and are not retentive of moisture. Blister is closely associated with cork and is probably produced by the same agencies.

It should be noted in this connection that the harmful effects of drouth are not always in proportion to the degree of desiccation. Other factors must be considered in a study of drouth troubles, and among these are the percentage of harmful substances in the soil water and the general growth condition of the plant.

SUMMARY

(1) Bitter-pit and Jonathan-spot are distinguished from rosy-aphis stigmonose, drouthspot, cork, and blister. Bitter-pit usually appears first as spots of dead, brown tissue in the subepidermal portion of the apple. These spots are associated with the terminal branches of the vascular bundles and in later stages of the disease the browning often follows the vasculars deep into the flesh of the apple. Rosy-aphis stigmonose is characterized by similar brown spots in the subepidermal region

but the affected tissue is firmer than in the case of bitter-pit and there is no association with the vascular bundles. The early stages of Jonathan-spot are confined to the color-bearing cells of the skin of the apple. Drouthspot is characterized by the checking of the growth at certain points on the apple without the production of any large quantity of corky tissue. Cork differs from the drouthspot in the presence of comparatively large spots of brown corky tissue and in the fact that these are usually rather deeply seated in the flesh of the apple. Blister is a superficial lesion associated with cork and characterized by its blister-like appearance.

(2) Drouthspot has been produced by sudden and extreme drouth. It has occurred on trees that were favorably located as well as on those that were growing under rather unfavorable soil conditions. Cork is apparently also a drouth effect, but it differs from drouthspot in the fact that its occurrence is usually associated with certain peculiar soil types.

(3) Experiments have shown that there is a close relationship between the soil-water supply of the orchard and the development of bitter-pit in storage. Heavy irrigation has greatly increased the disease, but not so much as medium irrigation followed by heavy irrigation. Light irrigation has greatly reduced it, but heavy irrigation followed by light has resulted in the lowest percentage of the disease. Sudden changes in the amount of soil water have apparently not increased the disease.

(4) Heavy irrigation may have been slightly favorable to the development of Jonathan-spot, but the contrasts have been too slight to justify definite conclusions.

(5) Large apples have shown greater susceptibility to bitter-pit than small ones, but with Jonathan apples heavy irrigation increased the disease on the medium-sized fruit as well as on the large, and with Grimes the percentage of increase from heavy irrigation has been even greater on small apples than on large ones. Apparently, large apples are not susceptible to bitter-pit merely because they are large, but rather because of certain conditions under which they become large.

(6) In 1915 there was more Jonathan-spot on the large apples than on the small ones, but in 1916 there seemed to be no correlation between size of fruit and severity of disease.

(7) During the first weeks of cellar storage there was always more Jonathan-spot developed on apples that were picked early than on apples that were picked late, but with longer periods of storage these contrasts seemed to largely disappear. The results indicate, however, a greater susceptibility in the early-picked fruit.

(8) Bitter-pit was worse on the Jonathan appies that were picked early than on those that were picked late.

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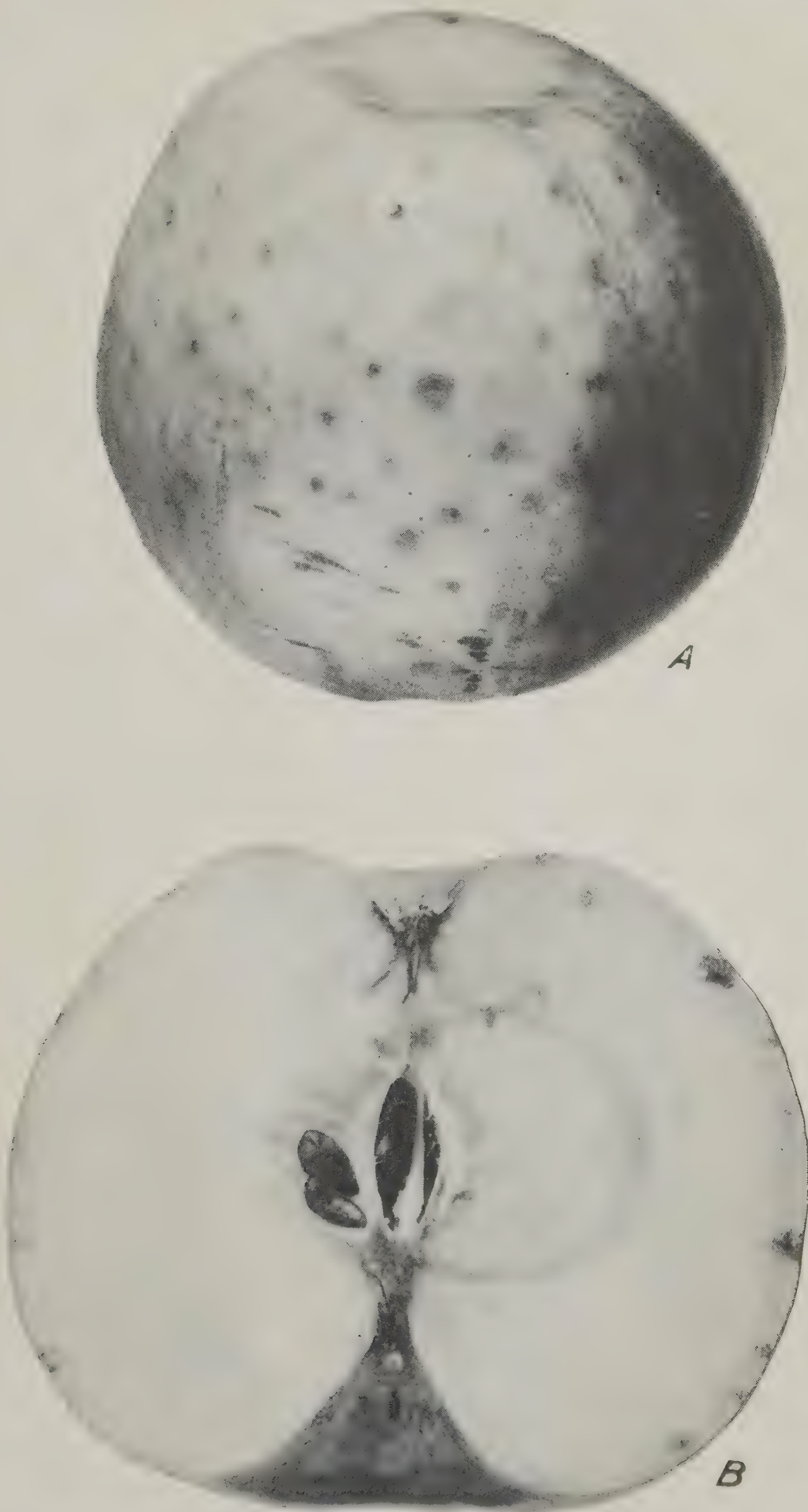
PLATE 2

A.—Early stage of bitter-pit on Northern Spy apple from Westminister, Vt., November 16, 1916.

B.—Cross section of the apple shown in A. Brown spots are evident just beneath the skin, and a few others can be seen deeper in the flesh of the apple.



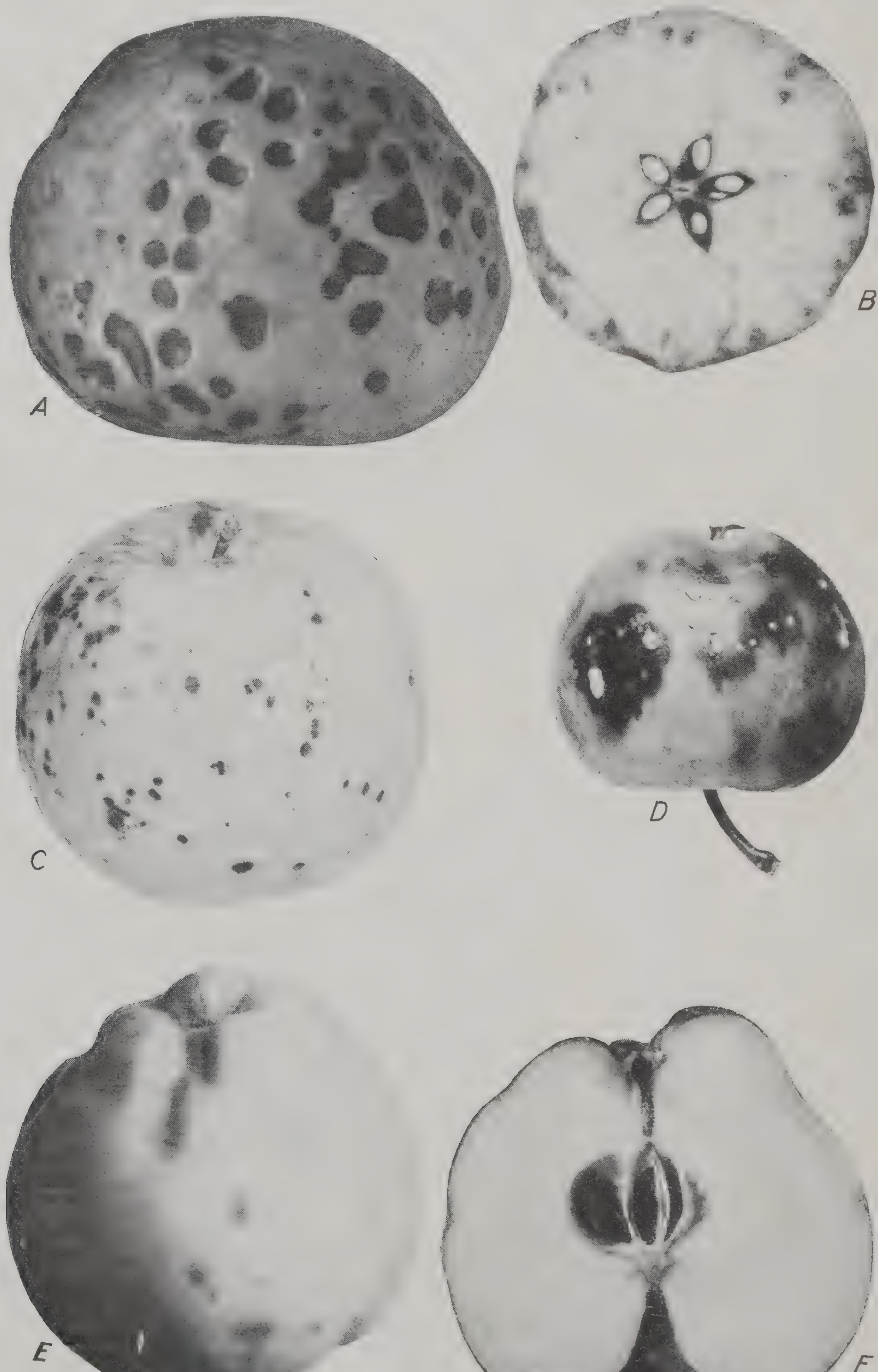


PLATE 3

A.—Late stage of bitter-pit on Rhode Island Greening apple.

B.—Internal browning accompanying bitter-pit.

C.—Jonathan-spot on Jonathan apple.

D.—Early stage of drouthspots on a Winesap apple from Wenatchee, Wash. The drops of exudate can be seen on the surface of the apple.

E.—Late stage of drouthspots on a Winesap apple. Note the deep depressions scattered over the surface of the apple.

F.—Cross section of the apple shown in E. Note the almost entire absence of brown corky tissue.

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PLATE 4

A.—An apple orchard showing the furrow system of irrigation employed in the experimental work at Wenatchee, Wash.

B.—Jonathan apple tree showing the effects of drouth, Wenatchee, Wash. Photographed on September 1, 1915.


A
B

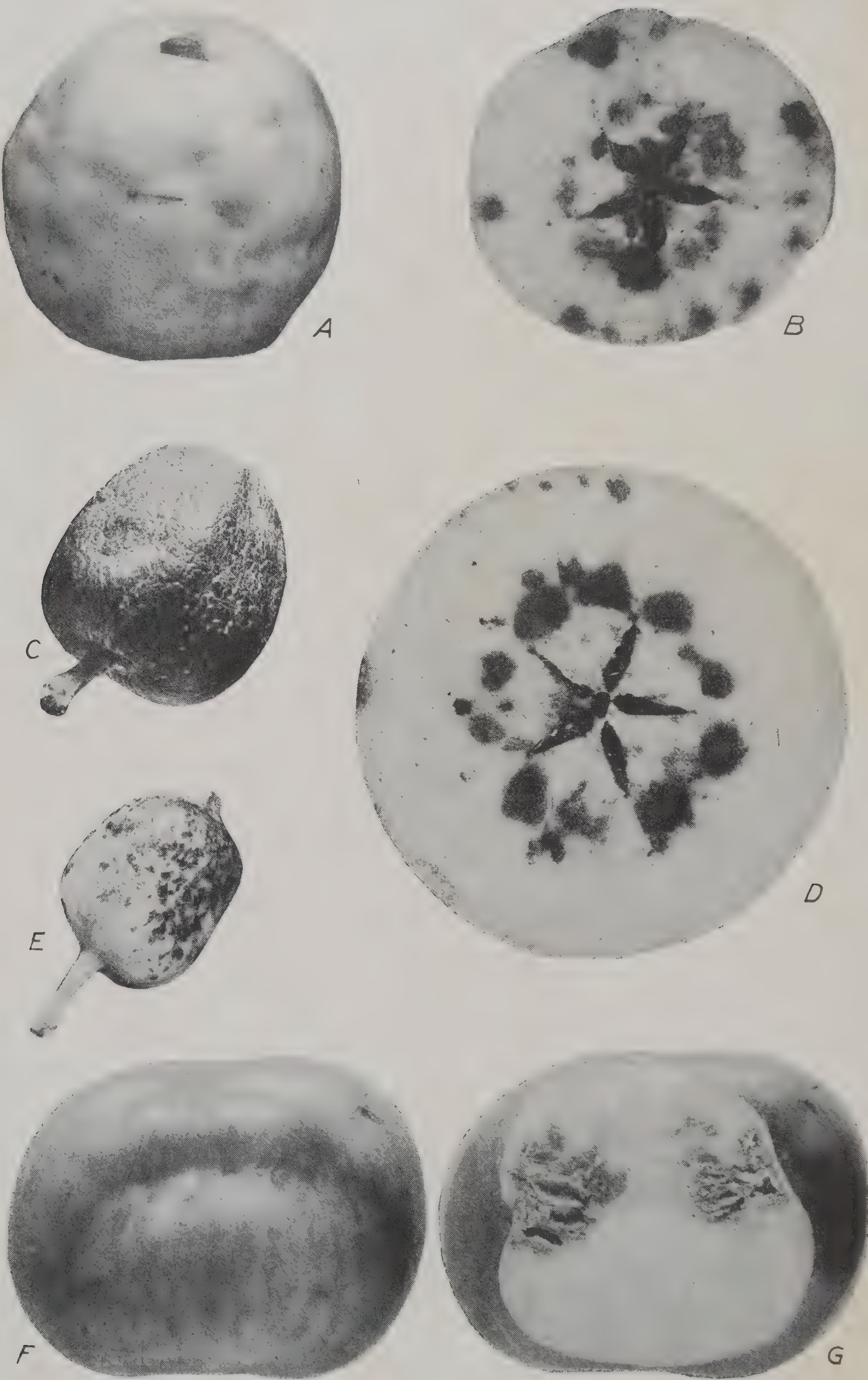
A
B
C
D
E
F
G

PLATE 5

A.—Cork on Yellow Newtown apple from Hood River, Oreg. Note the roughene, appearance.

B.—Cross section of the apple shown in A. Note the area of brown corky tissue.

C.—White Pearmain apple showing the severity of the 1915 drouth at Wenatchee, Wash. No drouthspots were developed on such apples.

D.—Cork, or "dryrot", on a King apple. Note the brown corky tissue near the core. In surface view such an apple appears normal.

E.—Blister on an Esopus apple from Entiat, Wash.

F.—An extreme case of Yorkspot on a York Imperial apple. Note the circular nature of the injury.

G.—Cross section of the apple shown in F. Note the pockets and the brown corky tissue beneath the surface depression.

RELATION OF CARBON DIOXID TO SOIL REACTION AS MEASURED BY THE HYDROGEN ELECTRODE[1]

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INTRODUCTION

In a previous article (*11*)[2] the authors have presented data concerning the question of soil reaction as determined by the hydrogen electrode. Since this work did not include direct measurements of the effect of carbon dioxid on the reaction of soils, it was thought desirable to carry out further experiments on this point. Before discussing the data obtained in these additional experiments it will be well to emphasize again the fundamental principles upon which the conclusions of our first paper were based.

The present tendency to advance involved explanations of the nature of soil acidity seems to be unnecessary, for the simple conception of the relations of H- and OH-ion concentrations are in accord with the facts so far ascertained and are warranted by the accepted teachings of chemistry. The lack of agreement in the literature appears to be due to the attempt to use interchangeably the terms "lime requirement" and "soil acidity." In the methods of determining the lime requirement it is proposed to measure the amount of lime required to bring the soil to an end point dependent upon arbitrarily selected conditions. These methods are in themselves so varied and the final measurement of reaction so difficult, that the discordant results which appear in the literature are wholly to be expected.

On the other hand, the term "soil acidity" has a definite and precise meaning—namely, that condition of the soil in which its aqueous solution contains H ions in excess of OH ions. In our opinion it would be preferable to refer to soil acidity, soil neutrality, and soil alkalinity as those phases of soil reaction in which the H-ion concentration is respectively greater than, equal to, or less than the OH-ion concentration. These H- ion concentrations may be definitely determined by measurements with the hydrogen electrode.

The lime requirement, in so far as it is related to soil acidity, would consist of that amount of lime necessary to bring an acid soil to the neutral point as ascertained by the above-mentioned procedure. Such a lime requirement implies that the dissolved and total undissolved soil acids have been neutralized. To put this procedure into practice may involve

[1] From the Divisions of Agricultural Chemistry and Soil Chemistry and Bacteriology in equal cooperation.

[2] Reference is made by number (italic) to "Literature cited," p. 147-148.

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certain inherent difficulties. The reaction is so prolonged either by the slow rate of solution of the soil acids or their slow diffusion through the soil particles that the point of neutrality may not be easy to establish and maintain permanently. Thus an apparent state of equilibrium at the neutral point may be attained with a subsequent slow return to an acid condition, owing to the solution and diffusion of the soil acids. But once the total soil acids have been neutralized, a further return to an acid condition can come about only through leaching processes or, possibly, in a few cases through decomposition of organic matter.

As pointed out, some soils whose solutions are neutral or alkaline remove considerable quantities of calcium hydroxid from the solution without materially increasing the OH-ion concentration of the soil suspension. In certain cases this reaction might be erroneously attributed to the soil acids. Although the term "lime requirement" is in common usage in agricultural literature, it has been variously interpreted by different investigators. At present the term is devoid of scientific significance. In distinction thereto soil reaction whether acid, neutral, or alkaline, is capable of precise definition and determination.

EFFECT OF CARBON DIOXID ON SOIL REACTION

In considering the effect of carbon dioxid on soil reaction MacIntire (*8*) states that many acid soils when extracted with water saturated with carbon dioxid yield alkaline extracts. He also makes the following conclusion:

> Since we admit that the soil solution is the medium through which a plant absorbs its mineral supply, we are compelled to conclude that a plant's source of nutrition is almost always alkaline, but of varying degrees of alkalinity.

From the nature of the chemical equilibria involved (*2*, *9*, *4*), we have been unable to reach the conclusion that a solution existing in contact with an acid soil can ever become alkaline owing to any change in the partial pressure of carbon dioxid. An increase in carbon-dioxid tension would either be without effect upon the H-ion concentration, or else would increase it, depending upon the relative dissociation constants of carbonic acid and the soil acids. It is conceivable that certain acid soils when extracted with water saturated with carbon dioxid might yield filtrates which would give an alkaline reaction after their carbon-dioxid content had come into equilibrium with the partial pressure of atmospheric carbon dioxid, but the equilibria governing the reaction of the soil solution in contact with the soil are by no means identical with those regulating the reaction of the filtrate obtained from such a soil.

In order to obtain direct evidence on the influence of carbon dioxid on soil reaction, the experiments reported in this paper were undertaken. The method of procedure was similar to that previously described by the authors, with the addition of an arrangement for controlling the partial pressure of carbon dioxid in the atmosphere above the soil. By adopting

a simplified form of the apparatus used by McClendon and Magoon (7) and McClendon (6) for investigating the H-ion concentration of sea water, we have been able to obtain the desired data.

DESCRIPTION OF APPARATUS AND RESULTS OBTAINED

The hydrogen-electrode apparatus was the same as that previously described in this journal. To provide a chamber for mixing the hydrogen and carbon dioxid a graduated 1,000-c. c. cylinder, the base of which had been cut off, was immersed in a larger cylinder filled with mercury. The upper end of the inner cylinder was tightly stoppered and contained two capillary stopcocks for admission and outlet of the gases. A definite quantity of purified hydrogen, electrolytically generated, was admitted to this cylinder through one stopcock. Through the other stopcock there was admitted from a gas burette a known quantity of pure carbon dioxid. Both gases were measured at atmospheric pressure. A sufficient time was then allowed for the thorough diffusion of the gases, which was aided by raising and lowering the inner cylinder. The reservoir of mixed gases was then connected to the hydrogen-electrode chamber which contained the soil suspension. Forty to seventy c. c. of the gas mixture were forced into the space above the soil suspension, adjusted to atmospheric pressure, and the hydrogen-electrode cell was then closed. Equilibrium was hastened by the shaking method, and the voltmeter readings were noted. This procedure was repeated with new portions of the gas mixture until the voltmeter readings were constant to within 0.005 volt. The experimental details and results are recorded in Table I.

The term "With previous carbonation" signifies that carbon dioxid has been passed through the soil suspension for a period of ½ to 2 hours previous to the determination of the H-ion concentration. The purpose of this step was to ascertain the effect of thoroughly saturating the soil with carbon dioxid upon its subsequent reaction. This also proved to be necessary in the case of some of the alkaline soils to insure complete saturation of the carbonates present, thus making it possible to attain the final equilibrium when using the smaller percentages of carbon dioxid, without the preparation of excessive quantities of the gas mixture. By satisfying in this manner the capacity of the soil to combine with carbon dioxid, it is possible to reach a partial pressure of carbon dioxid above the suspension in the hydrogen-electrode cell comparable to that in the mixing cylinder.

As expressed in Table I the 0 per cent of carbon dioxid means that several portions of pure hydrogen were used to obtain equilibrium. With such a technic the loss of carbon dioxid from the soil is minimized. Thus, the atmosphere above the suspension will undoubtedly contain a small percentage of carbon dioxid.

TABLE I.—*Effect of carbon dioxid on the reaction of soil suspensions*

Soil No.	Description of soils.	Quantity of soil.	Quantity of water.	Percentage of carbon dioxid in gas mixture by volume.	Reaction without previous carbonation.		Reaction with previous carbonation.	
					Readings on voltmeter.[a]	H-ion concentration.	Readings on voltmeter.[a]	H-ion concentration.
		Gm.	*C. c.*			*Gram-molecules per liter.*		*Gram-molecules per liter.*
1	Fine sandy loam (California)	10	50	0.00	0.589	0.40×10^{-4}	0.592	0.37×10^{-4}
1	do			.42	.590	$.40\times10^{-4}$		
1	do			1.90			.589	$.40\times10^{-4}$
1	do			9.00	.580	$.59\times10^{-4}$		
2	Silty clay loam (California)	10	50	.00	.783	$.17\times10^{-7}$	.763	$.38\times10^{-7}$
2	do			.22			.742	$.92\times10^{-7}$
2	do			.42	.747	$.73\times10^{-7}$		
2	do			.49			.744	$.85\times10^{-7}$
2	do			1.90	.726	$.18\times10^{-6}$	.726	$.18\times10^{-6}$
2	do			4.80	.709	$.34\times10^{-6}$		
2	do			9.00	.688	$.80\times10^{-6}$		
3	Clay loam (California)	10	50	.00	.896	$.20\times10^{-9}$		
3	do			.42			.773	$.26\times10^{-7}$
3	do			1.90			.768	$.33\times10^{-7}$
3	do			4.80	.753	$.59\times10^{-7}$		
3	do			9.00	.762	$.42\times10^{-7}$	.756	$.53\times10^{-7}$
4	Clay loam (Pennsylvania)	10	50	.00	.700	$.49\times10^{-6}$	.712	$.31\times10^{-6}$
4	do			.22	.687	$.84\times10^{-6}$		
4	do			1.90			.685	$.90\times10^{-6}$
4	do			9.00	.665	$.20\times10^{-5}$		
5	Clay loam (Louisiana)	10	50	.00	.667	$.18\times10^{-5}$	.665	$.20\times10^{-5}$
5	do			4.80	.644	$.46\times10^{-5}$	.647	$.40\times10^{-5}$
6	Silty clay loam (Wisconsin)	10	50	.00	.658	$.26\times10^{-5}$	.663	$.21\times10^{-5}$
6	do			1.90			.653	$.32\times10^{-5}$
7	Clay loam (Louisiana)	10	50	.00	.684	$.94\times10^{-6}$	.685	$.90\times10^{-6}$
7	do			1.90			.664	$.21\times10^{-5}$
8	Silty loam (California)	10	50	.00	.604	$.23\times10^{-4}$	.602	$.25\times10^{-4}$
8	do			1.90	.603	$.24\times10^{-4}$		
9	do	10	50	.00	.792	$.13\times10^{-7}$	.789	$.14\times10^{-7}$
9	do			.20			.770	$.30\times10^{-7}$
9	do			2.00			.726	$.18\times10^{-6}$
10	Silty clay loam (California)	10	50	.00	.770	$.30\times10^{-7}$		
10	do			.20			.764	$.38\times10^{-7}$
10	do			.42			.758	$.49\times10^{-7}$
10	do			1.90			.724	$.19\times10^{-6}$
10	do			4.90			.700	$.49\times10^{-6}$
11	do	10	50	.00	.782	$.19\times10^{-7}$		
11	do			.50			.755	$.55\times10^{-7}$
11	do			1.90			.735	$.12\times10^{-6}$
11	do			4.90			.718	$.24\times10^{-6}$

[a] Mercury cell, with mercuric chlorid and potassium chlorid in *N/10* concentration.

Since the measurement of the H-ion concentration is based on hydrogen at atmospheric pressure, any diminution in this pressure caused by the admixture of carbon dioxid would result in a certain lowering of the electromotive force. Loomis and Acree (5) have determined the changes in electromotive force resulting from the diminution of the partial pressure of hydrogen within certain ranges. Their data indicate that these changes are so slight as to be without significance in the present investigation, hence no corrections for this factor have been made. Most of the determinations reported above are the averages of duplicates. The agreement of these duplicates was in nearly all cases within 0.005 volt.

The data in the above table have to do with the effect of carbon dioxid on three general types of soil reaction. The acid type of reaction is

represented by soils 1, 4, 5, 6, 7, and 8, the neutral or slightly alkaline type by soils 2, 9, 10, and 11, and the strongly alkaline reaction by soil 3.

Considering first the case of the acid soils, we note that the increase in H-ion concentration of the soil suspensions, in contact with even the higher percentages of carbon dioxid, is scarcely greater than the errors of observation. In soils 1 and 8, whose suspensions give H-ion concentrations of the magnitude 10^{-4}, there has been no perceptible change in reaction due to the presence of carbon dioxid. The other acid soils, which have a smaller concentration of H ion, show some slight increase in acidity when in contact with the mixture of gases containing carbon dioxid. This might be expected from a consideration of the relation of the dissociation of carbonic acid to that of the soil acids. Those soils whose acids dissociate comparably to carbonic acid are not measureably affected by the partial pressures of carbon dioxid used in these experiments, while soils containing less dissociated acids have their H-ion concentration increased to a slight extent by the carbon dioxid.

The H-ion concentration of suspensions of the slightly alkaline soils is appreciably increased by increasing the partial pressure of the carbon dioxid. The degree of increase in acidity seems to be dependent upon the proportion of carbon dioxid in the gas mixture. In fact, the higher percentages of carbon dioxid brought about a slightly acid reaction in the suspensions of these soils.

In case of soil 3, a so-called "alkali" soil, which had a very low H-ion concentration, a large increase was caused by the introduction of carbon dioxid into the hydrogen-electrode cell.

DISCUSSION OF RESULTS

The results recorded in this paper show that the effect of carbon dioxid on the H-ion concentration of soil suspensions is not an insurmountable difficulty in obtaining the reaction of soils under various conditions by means of the hydrogen electrode. The technic followed in the former investigation in which the loss of carbon dioxid is minimized, although not entirely avoided, evidently gives results of the same order of magnitude as would be obtained if there were no loss of carbon dioxid. The adoption of such a view is warranted by the fact that the maintenance of a small partial pressure of carbon dioxid above the soil suspension in the hydrogen-electrode cell, only altered the H-ion concentration by less than a magnitude. It should be remarked, however, that in soils containing alkali carbonates, and having a high OH-ion concentration, the partial pressure of the carbon dioxid exercises a very pronounced effect upon the reaction, as instanced by soil 3. For a clear exposition of equilibria governing such systems the reader is referred to Johnston (*4*).

So far we have not considered the question of H-ion concentration in soils under field conditions. By determining the carbon-dioxid content of the soil atmosphere under field conditions and then duplicating the

partial pressure of carbon dioxid in the manner suggested in this paper it should be entirely practicable to obtain a measurement of the H-ion concentration identical with that of the soil in the field. Russell and Appleyard (*10*) have found that the carbon-dioxid content of the soil atmosphere under different conditions varied between 0.02 and 2 per cent, the general mean of arable soils being 0.25 per cent. If these percentages of carbon dioxid are to be regarded as typical for field soils, then in view of the present experiments the changes in carbon-dioxid content during sampling and laboratory manipulation would not invalidate our inferences with regard to the reaction of soils under natural conditions.

The a priori considerations already presented in the first portion of this paper with respect to the effect of carbon dioxid on the reaction of soils are entirely substantiated by the experimental data, which lead to conclusions at variance with those of MacIntire (*8*) on this point. Even saturating the soil suspension with carbon dioxid previous to measuring the reaction did not decrease the H-ion concentration. Therefore it follows that an acid soil would never present to the plant a soil solution of alkaline reaction, notwithstanding any increase in the partial pressure of carbon dioxid. It should be recalled that the criteria heretofore used for judging the reaction of soils do not always permit of an accurate distinction between soils of different reactions. Some soils may be judged as acid from the standpoint of certain lime requirement methods, when in reality their reaction may be alkaline. For this reason it may be doubted whether some of the soils reported as yielding alkaline extracts were in fact acid. Although in certain instances the application of lime may be followed by an increased crop yield, this result may not be dependent upon any change in the reaction of the soil due to the addition of lime. The more accurate interpretation of liming experiments demands that an attempt be made to differentiate lime as a neutralizer of acidity and the other directly or indirectly beneficial effects of lime on the soil or the plant.

The statement frequently met in the literature that the extracts from soils considered acid have a neutral or alkaline reaction has led to the conclusion that water-soluble acids are not found in acid soils. We desire to emphasize again the point that extracts of acid soils, especially those prepared with carbonated water, might become neutral or alkaline after the loss of carbon dioxid from the extract. Moreover the reported results on the extracts are likely to be misinterpreted, for they do not take into account the H-ion concentration but are based upon titrations, using indicators whose end points may be removed from neutrality by several magnitudes. Indeed Gillespie (*3*) has found that soils determined to be acid by the hydrogen electrode, yield extracts whose reactions, colorimetrically estimated, were in close agreement with the hydrogen-electrode measurements.

One of Bouyoucos's (*1*) conclusions from his valuable and ingenious application of the freezing-point method to soil investigations is as follows:

> Since no mineral soil out of a great number tested gave an acid curve but only an absorption curve, and inasmuch as the free acid, and acid salt produced in these soils when they were treated with neutral salts, or acid and acid salts, were carried away by washing and the soils then gave an absorption curve, the conclusion seems to be that the presence of soluble acids, or acid salts, in the mineral soils under favorable natural conditions is only temporary, if ever present, and never permanent. The acidity or lime requirement of soils, therefore, seems to be due mainly to the insoluble acids of the soil, the silicic acid, silica, acid alumino-silicates, and perhaps to the insoluble organic matter. There appears to be then practically no active acidity in the mineral soils, but only negative. Exceptions to these general statements are probably very few.

Contrary to the above conclusion, the data presented in this and other papers (*3*, *11*), show that the solution in equilibrium with the soil particles of certain soils contains H ions in excess of OH ions. Such soils are therefore necessarily acid and they include various types, many of which would be called mineral soils. Furthermore, it is well to bear in mind that the hydrogen electrode is capable of measuring specifically the H-ion concentration, while the freezing-point method is unsuited to this purpose. This is especially true in dealing with such heterogeneous systems as those of the soil mass. These statements are not to be construed as denying the possible value of the freezing-point method in estimating the total "lime requirement."

The soil acids are frequently referred to as insoluble, but such insolubility is, as a matter of fact, only relative, for complete insolubility is practically unknown and the soil acids must therefore have a definite solubility although it may be slight. The important consideration is that the solution of an acid soil must be continuously acid in just the same way that a solution in contact with silicic acid or other slightly soluble acids is always acid. It is quite true that when the soil is treated with a base in order to bring about a condition of alkalinity, by far the greater part of the base is used in combining with acids which at any given moment were not in the solution. But this is in no way opposed to the conclusion that the solution in contact with an acid soil is an acid solution and would accordingly offer an acid medium for plant growth. The effect of such a medium would be related to its H-ion concentration and not to the total quantity of base required to neutralize all the soil acids present. In other words, the reaction of a soil is concerned with the dissolved fraction, which is in equilibrium with the undissolved soil mass. If any added substance—for example, calcium carbonate—disturbs this equilibrium, then it is clear that the undissolved portion of the soil will enter into the reaction in accordance with the laws of mass action.

EFFECT OF POTASSIUM CHLORID ON THE H-ION CONCENTRATION OF SOILS

As previously shown, neutral salts added to suspensions of certain soils considerably increased their H-ion concentration. This fact has brought up the question as to the effect of the diffusion of potassium chlorid from the agar connecting tube into the soil suspension. In order to determine the magnitude of the possible changes induced in the reaction of soil suspensions by the escape of potassium chlorid into them, several experiments were undertaken. For this purpose it was necessary to eliminate as far as possible the diffusion of potassium chlorid into the soil suspension. As shown in Table II, one procedure consisted in bringing the soil suspension into equilibrium with hydrogen without any possible chance for contamination with potassium chlorid, then the electromotive force was read just as the agar tube touched the suspension, thus reducing the diffusion of the potassium chlorid to a minimum. This reading was then compared with subsequent readings made in the manner heretofore described. In addition, some agar tubes prepared with soil extracts were substituted for the potassium chlorid tubes. The sensitivity of our galvanometer did not allow of a greater accuracy than 0.02 volt when the soil-extract tubes were used.

TABLE II.—*Effect of potassium chlorid on the reaction of soil suspensions*

Laboratory number of soil.	With first contact of potassium chlorid tube.		After many contacts of potassium chlorid tube.		Soil-extract tube.	
	Readings on voltmeter.	H-ion concentration.	Readings on voltmeter.	H-ion concentration.	Readings on voltmeter.	H-ion concentration.
		Gram-molecules per liter.		*Gram-molecules per liter.*		*Gram-molecules per liter.*
7	0.681	0.11×10^{-5}	0.664	0.21×10^{-5}	0.661	0.23×10^{-5}
25	....	....	.764	$.38 \times 10^{-7}$	.771	$.29 \times 10^{-7}$
1	.595	$.33 \times 10^{-4}$	.589	$.40 \times 10^{-4}$	....	....
26	.677	$.12 \times 10^{-5}$	.675	$.13 \times 10^{-5}$	....	....
27	.653	$.32 \times 10^{-5}$	.651	$.35 \times 10^{-5}$	....	....
28	.678	$.12 \times 10^{-5}$	.675	$.13 \times 10^{-5}$	....	....
29	.702	$.46 \times 10^{-6}$	.699	$.51 \times 10^{-6}$	....	....
31	.610	$.18 \times 10^{-4}$	.608	$.19 \times 10^{-4}$	....	....
32	.739	1.0×10^{-7}	.738	$.11 \times 10^{-6}$	....	....
33	.660	$.24 \times 10^{-5}$	.660	$.24 \times 10^{-5}$	....	....
34	.653	$.33 \times 10^{-5}$	.653	$.33 \times 10^{-5}$	....	....

It is evident from the data in Table II that the slight diffusion of potassium chlorid from the agar tube has a tendency to increase the H-ion concentration of the soil suspension. In almost all cases this increase corresponds to less than 0.005 volt. For most agricultural purposes this difference has no significance. By bringing the entire system into equilibrium with hydrogen before immersing the agar tube and by keeping the tube out of the suspension except momentarily at the time of reading the electromotive force, it is believed that the error will be entirely negligible.

SUMMARY

(1) The H-ion concentrations of soil suspensions have been measured under various partial pressures of carbon dioxid.

(2) The H-ion concentration of suspensions of acid soils is not markedly affected by increasing the content of carbon dioxid up to 10 per cent. The H-ion concentration of slightly alkaline soils is slightly increased by such treatments. A notable increase in H-ion concentration is observed when soils containing alkali carbonates are similarly treated.

(3) It has not been found that any treatment with carbon dioxid can produce an alkaline reaction in the suspension of an acid soil.

(4) When the original conditions are restored, no permanent change in soil reaction could be attributed to the carbon dioxid.

(5) Further experiments with the hydrogen electrode have confirmed the point of view that solutions in equilibrium with acid soils contain H ion in excess of OH ion.

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A STUDY OF THE PLOW BOTTOM AND ITS ACTION UPON THE FURROW SLICE[1]

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INTRODUCTION

The most ancient records show that from a very remote period man has used the plow, in one form or another, to assist him in stimulating the earth to bring forth a more bountiful harvest. As has been the case in many other lines of endeavor, theory has trailed far behind observation and experience in developing this implement. In fact, as far as can be ascertained, it was not until the last half of the eighteenth century that any serious attempt was made to develop a plow bottom from a theoretical standpoint, and even then the productions of Jefferson, Lambruschini, Small, Rham, and others can not be considered as thoroughly grounded upon well-developed theories; rather their works should be looked upon as hypotheses (fig. 1). Experience in the field generally proved that the machines designed by these men were not all that could be desired—for example, it is reported[3] that when Lambruschini's helicoidal moldboard was taken into the field for trial the driver of the draft animals immediately observed that the force required to move this plow was too great for the results obtained. To be sure, geometrically exact moldboards furnished the basis in many instances for more perfect developments, but the results obtained by empirical plow designers who worked in the field were so far superior to the results obtained by the men who worked in the laboratory that the theorists were soon completely outstripped and even held up to ridicule by the men who developed their machines in the hard school of experience, until at the present time we find special types of plow bottoms designed

[1] Approved for publication in the Journal of Agricultural Research by the Director, Cornell University Agricultural Experiment Station.

[2] The experimental work for this paper was done under the direction of Prof. H. W. Riley, of the Department of Rural Engineering, Cornell University, and the mathematical developments were prepared under the supervision of Prof. F. R. Sharpe, of the Department of Mathematics. In addition to the above, grateful acknowledgments are given to the following: To Profs. James McMahon and Virgil Snyder, of the Department of Mathematics, for their most timely and helpful suggestions; to Mr. J. E. Reyna, Instructor in Drawing, College of Agriculture, Cornell University; and to Mr. L. S. Baldwin, Instructor in General Engineering Drawing, University of Illinois, for making the drawings.

[3] LAMBRUSCHINI, R. D'UN NUOVO ORECCHIO DA COLTRI. *In* Gior. Agr. Toscano, v. 6, p. 37–80. 1832.

to meet certain field conditions; but no well-developed theory is available to serve as a guide in this work.

This paper is an attempt to begin a fundamental analysis of the plow bottom and its work, in the hope that some light may be thrown upon the theory of this humble but perplexing machine, and other attempts stimulated to delve further into the secrets which are still to be revealed regarding the theory of this important implement. Empirical methods have given the world plow bottoms which work well. It is still to be hoped that scientific investigation can refine and further perfect, supplement as it were, the productions of experience.

The work undertaken by the writer can be naturally divided into three parts: (1) A study of the forms of plow bottoms; (2) an attempt to

Date	Name	Generatrix	Directrixes	Equation of Surface
	Small	Straight Line	Straight Line & Catenary	
	Stephens	Straight Line	Straight Line and arc of Circle	$\frac{y}{x} = \tan[f(z)]$
1788	Jefferson	Straight Line	Straight Lines	$\frac{x^2}{a^2} - \frac{y^2}{b^2} = 2nz$
1818	Davis	Arc of Circle	Arcs of Circle	
1832	Lambruschini	Straight Line	Straight Line and Helix	$\frac{y}{x} = \tan(az)$
1839	Witherow and Pierce	Arc of Cycloid	Arcs of Cycloid	
1840	Rham	Straight Line	Straight Lines	$\frac{x^2}{a^2} - \frac{y^2}{b^2} = 2nz$
1840	Rham	Straight Line	Curves	
1852	Knox	Straight Line	Arcs of Circles	Ruled surface of eighth order
1854	Gibbs	Straight Line	Arcs of Circles	$\frac{x^2}{r^2} + \frac{y^2}{r^2} - 1 = 0$
1863	Mead	Straight Line	Arcs of Circles	$\frac{x^2}{a^2} + \frac{y^2}{b^2} - \frac{z^2}{c^2} = 0$
1867	Holbrook	Straight Line	Straight Lines	$\frac{x^2}{a^2} + \frac{y^2}{b^2} - \frac{z^2}{c^2} = 1$
1834	Jacobs	A portion from each of 2 Surfaces; each surface having 2 sets of straight line generators.		

FIG. 1.—Diagram giving the generatrices, directrices, and equations of surfaces of historical plow bottoms.

analyze the motion of the soil particles as they pass over the surface, and (3) a mathematical analysis of the surfaces of the most important historical plow bottoms which were designed to be geometrically exact. It was, and still is, hoped that a knowledge of just what the plow bottom is and how it performs its work will be of material assistance in developing a theory which will furnish a very definite basis for the proper design of this fundamental implement of tillage.

FORMS OF THE PLOW BOTTOM

A study of modern American-manufactured plow bottoms reveals the fact that a large number of these are so constructed that their surfaces contain sets of straight lines, each set consisting of an infinite number of straight lines, so related that an equation or equations satisfied by the coordinates of points on the surface can be found.

Plate 6, A, represents a bottom with two sets of straight lines. The few lines shown in the illustration indicate that through every point of the surface two straight lines can be drawn which lie wholly on the surface until they pass off the edges of the bottom. These straight lines furnish the basis for the proof that such a surface is a portion of an hyperboloid of one sheet (for the form of this surface see fig. 3 to 7) whose equation can be developed and studied with mathematical exactness. The method of developing this equation will be given later, but at present we are mainly interested in the fact that there is a classs of plow bottoms on whose surfaces lie sets of straight lines, and, further, that one equation can be developed which will approximately represent the working surface of such a bottom.

Further study shows that the surfaces of other plow bottoms contain sets of straight lines, but that one equation will not completely describe such a surface. In Plate 6, B, a bottom is shown whose surface is composed of a portion of each of two surfaces. Plate 6, C, shows a similar bottom, but in this case the two surfaces merge into each other farther back upon the moldboard.

In Plate 6, D, a class of bottoms is represented whose entire surfaces do not contain an infinite set of straight lines. It is true that the share and back end of the moldboard exhibit the same characteristics that the first two classes have shown, but the lines do not continue to the fore part of the moldboard.

Plate 7, A, shows a plow bottom with a convex surface which has two sets of straight lines.

The American-manufactured plow bottoms studied can thus be divided into three general classes: (1) A portion of one quadric surface; (2) a portion of each of two quadric surfaces, and (3) nonquadric surfaces. Nearly all forged bottoms belong to classes 1 and 2 with the majority falling into class 2, while most of the cast bottoms belong to class 3. It should be noted, however, that some recently designed cast bottoms depart from the general characteristics of class 3 and show clearly the two quadric surfaces of class 2. The lines running in the general direction, front to rear, marked "*l*," (Pl. 6, A) will be called longitudinal lines, and those running in the general direction, top to bottom, marked "*t*" (Pl. 6, A) will be called transverse lines.

For the purpose of studying the forms of the various surfaces under consideration, a machine, illustrated in Plate 7, B, was designed and built for measuring the space coordinates of any desired point.[1] By means of slots and a system of pulleys attached to the drafting board the cross-bar can be kept horizontal and be moved both laterally and vertically, while the drafting board is attached to a frame which can be moved

[1] Similar machines are described in the following publications: GOULD, J. S., et al. REPORT ON TRIALS OF PLOWS. *In* Trans. N. Y. State Agr. Soc., v. 27, pt. 1, 1867, p. 426. 1868.

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backward and forward upon guides so marked that the board in all positions will be squarely across the guides. When a plow bottom is properly placed upon the platform the x, y, and z coordinates of any point upon the surface can thus be recorded upon coordinate paper fastened upon the drafting board.

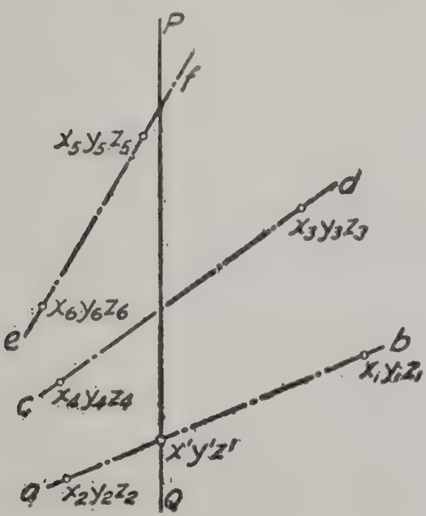


FIG. 2.

DEVELOPMENT OF THE EQUATION

From a mathematical standpoint the surface shown in Plate 6, A, presents the problem of finding the equation of a surface, given two sets of straight-line generators. This can be done if the equations of any three lines in the same set are known. Select three lines *ab*, *cd*, and *ef* (fig. 2). Let x_1, y_1, z_1, and x_2, y_2, z_2 be the coordinates of two points upon line ab; x_3, y_3, z_3, and x_4, y_4, z_4 of two points upon line *cd;* and x_5, y_5, z_5, and x_6, y_6, z_6 of two points upon line *ef*.

The equations of the lines *ab*, *cd*, and *ef*, are

$$\frac{x-x_1}{x_2-x_1}=\frac{y-y_1}{y_2-y_1}=\frac{z-z_1}{z_2-z_1}; \tag{1}$$

$$\frac{x.-x_3}{x_4-x_3}=\frac{y-y_3}{y_4-y_3}=\frac{z-z_3}{z_4-z_3}; \tag{2}$$

and

$$\frac{x-x_5}{x_6-x_5}=\frac{y-y_5}{y_6-y_5}=\frac{z-z_6}{z_5-z_5}. \tag{3}$$

From (2) the following equation for a plane perpendicular to the XY-plane and containing the line *cd* is obtained:

$$u_4\equiv(x-x_3)\ (y_4-y_3)-(y-y_3)(x_4-x_3)=O. \tag{4}$$

Similarly from (2) the equation of a plane perpendicular to the YZ-plane and containing the line *cd* is

$$u_5\equiv(y-y_3)\ (z_4-z_3)-(z-z_3)\ (y_4-y_3)=O. \tag{5}$$

From (3), the equation of a plane perpendicular to the XY-plane and containing the line *ef* is

$$u_6\equiv(x-x_5)(y_6-y_5)-(y-y_5)(x_6-x_5)=O. \tag{6}$$

Similarly, from (3) the equation of a plane perpendicular to the YZ-plane and containing the line *ef* is

$$u_7\equiv(y-y_5)(z_6-z_5)-(z-z_5)(y_6-y_5)=O. \tag{7}$$

Consider

$$u_4 = Au_5. \tag{8}$$

where A is a constant. This is the equation of a plane which contains the intersection of planes (4) and (5); hence it contains the line cd.

Similarly

$$u_6 = Bu_7 \tag{9}$$

where B is a constant, is the equation of a plane which contains the line ef.

If A and B have such values that the point x', y', z' is on (8), (9), and (1), the line of intersection of (8) and (9) meets (1) and is a generator (see fig. 2). Hence,

$$A = \frac{(x'-x_3)(y_4-y_3)-(y'-y_3)(x_4-x_3)}{(y'-y_3)(z_4-z_3)-(z'-z_3)(y_4-y_3)}; \tag{10}$$

$$B = \frac{(x'-x_5)(y_6-y_5)-(y'-y_5)(x_6-x_5)}{(y'-y_5)(z_6-z_5)-(z'-z_5)(y_6-y_5)}; \tag{11}$$

and

$$\frac{x'-x_1}{x_2-x_1} = \frac{y'-y_1}{y_2-y_1} = \frac{z'-z_1}{z_2-z_1} = K; \tag{12}$$

where K is a constant.

From equations (12)

$$x' = K(x_2-x_1)+x_1 \tag{13}$$

$$y' = K(y_2-y_1)+y_1 \tag{14}$$

$$z' = K(z_2-z_1)+z_1 \tag{15}$$

From equations (10), (13), (14), and (15)

$$A = \frac{([K(x_2-x_1)+x_1-x_3](y_4-y_3))-([K(y_2-y_1)+y_1-y_3](x_4-x_3))}{([K(y_2-y_1)+y_1-y_3](z_4-z_3))-([K(z_2-z_1)+z_1-z_3](y_4-y_3))}; \tag{16}$$

and from equation (8)

$$A = \frac{(x-x_3)(y_4-y_3)-(y-y_3)(x_4-x_3)}{(y-y_3)(z_4-z_3)-(z-z_3)(y_4-y_3)}. \tag{17}$$

From equations (11), (13), (14), and (15)

$$B = \frac{([K(x_2-x_1)+x_1-x_5](y_6-y_5))-([K(y_2-y_1)+y_1-y_5](x_6-x_5))}{([K(y_2-y_1)+y_1-y_5](z_6-z_5))-([K(z_2-z_1)+z_1-z_5](y_6-y_5))}; \tag{18}$$

and from equation (9)

$$B = \frac{(x-x_5)(y_6-y_5)-(y-y_5)(x_6-x_5)}{(y-y_5)(z_6-z_5)-(z-z_5)(y_6-y_5)}. \tag{19}$$

Eliminating A, B, and K from (16), (17), (18), and (19), we have the equation of a surface through the lines ab, cd, and ef. The equations are left in this form because numerical substitutions are more easily made

at this point than would be the case if the indicated operations were first performed with the symbols.[1] The general form of the equation resulting from the previous operations is

$$ax^2+by^2+cz^2+2fyz+2gxz+2hxy+2lx+2my+2nz+d=O. \tag{20}$$

To reduce equation (20) to its simplest form the axes must be translated and rotated.

TRANSLATION OF AXES[2]

The origin of equation (20) is translated to the center by putting

$$x=x'+x_o, \qquad y=y'+y_o, \qquad z+z'+z_o; \tag{21}$$

he values of x_o, y_o, and z_o being obtained from the following:

$$ax_o+hy_o+gz_o+l=O \tag{22}$$

$$hx_o+by_o+fz_o+m=O \tag{23}$$

$$gx_o+fy_o+cz_o+n=O. \tag{24}$$

These substitutions give, after dropping the accents from x', y', and z', an equation of the following form:

$$ax^2+by^2+cz^2+2fyz+2gxz+2hxy+G=O; \tag{25}$$

where

$$G=lx_o+my_o+nz_o+d. \tag{25a}$$

ROTATION OF AXES[3]

Equation (25) can be further reduced by a rotation of the axes. This is accomplished by means of a cubic equation

$$k^3-(a+b+c)k^2+(ab+ac+bc-f^2-g^2-h^2)k-D=O; \tag{26}$$

where

$$D=\begin{vmatrix} a & h & g \\ h & b & f \\ g & f & c \end{vmatrix} \tag{26a}$$

Let the roots of (26) be k_1, k_2, and k_3. The desired equation, after translating and rotating the axes is

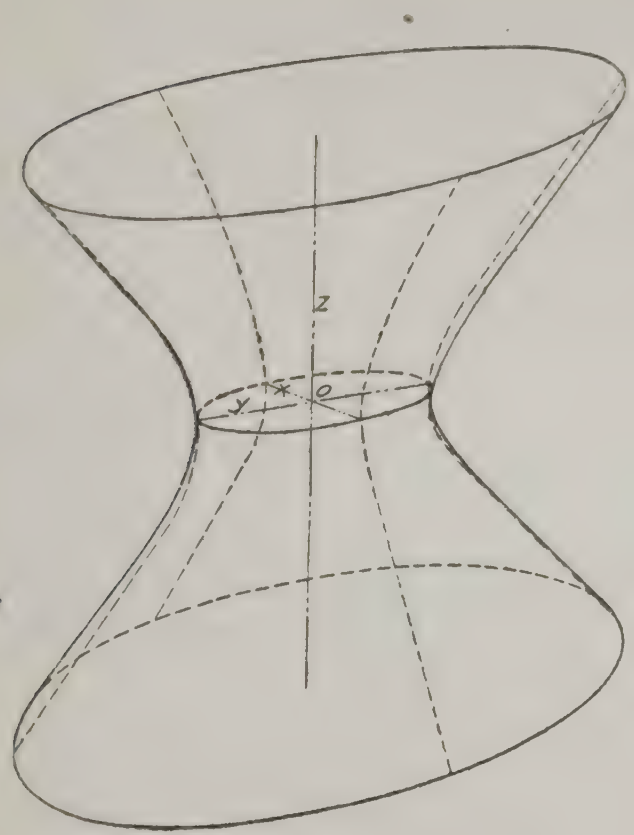
$$k_1x^2+k_2y^2+k_3z^2+\frac{\Delta}{k_1k_2k_3}=O;\text{[4]} \tag{27}$$

[1] A numerical problem is developed by this method upon pages 156 to 160.

[2] SNYDER, Virgil, and SISAM, C. H. ANALYTIC GEOMETRY OF SPACE,. p. 77. New York, 1914.

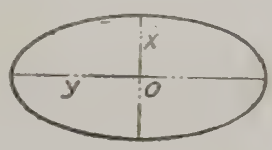
[3] Idem, p. 79.

[4] Idem, p. 86.



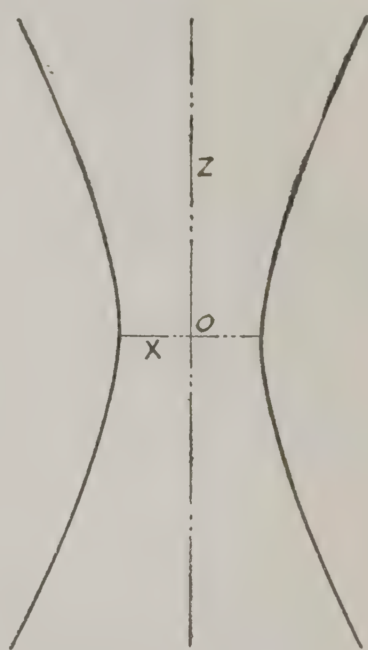
Skeleton. Hyperboloid of One Sheet

FIG. 3.



Section z=o, Fig. 3.

FIG. 4.

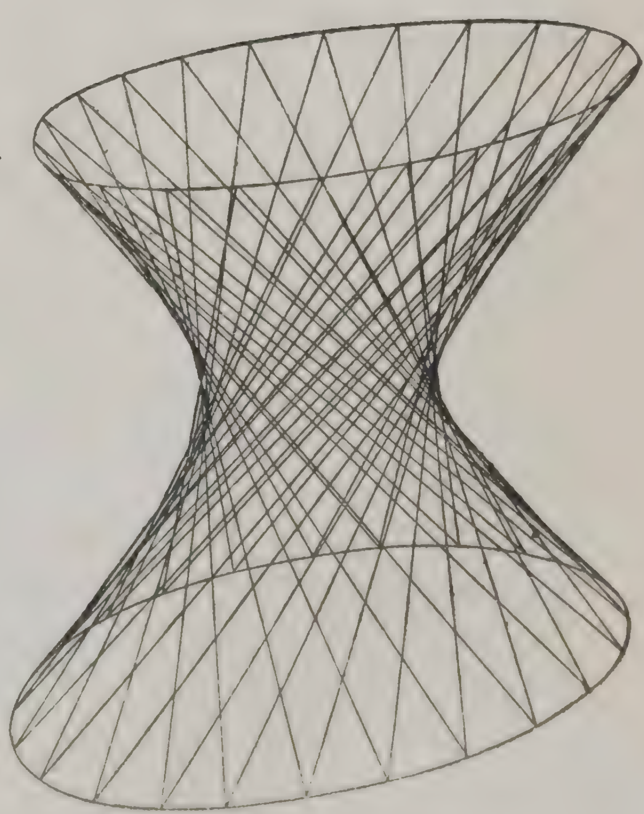


Section y=o, Fig. 3.

FIG. 5.

Section x=o, Fig. 3.

FIG. 6.



Hyperboloid of One Sheet, showing Lines upon the Surface

FIG. 7.

where $$\Delta = DG. \tag{27a}$$

The direction cosines λ, μ, v, of the angles which the new X-axis makes with the original axes are obtained from the following:

$$(a-k_1)\lambda + h\mu + gv = O \tag{28}$$

$$h\lambda + (b-k_1)\mu + fv = O \tag{29}$$

$$g\lambda + f\mu + (c-k_1)v = O \tag{30}$$

$$^2\lambda + \mu^2 + v^2 = 1. \tag{31}$$

Similarly, the direction cosines of the angles which the Y- and Z-axe make, after rotation, with the original axes are found by substituting k_2 and k_3, respectively, for k_1 in equations (28), (29), (30), and (31).

When equation (27) was developed from the surface of a plow bottom having two sets of straight-line generators, it had the following general form:

$$\frac{x^2}{a^2} + \frac{y^2}{b^2} - \frac{z^2}{c^2} = 1 \text{ [1]} \tag{32}$$

This is the equation of an hyperboloid of one sheet, a vase-shaped figure, the skeleton of a section of which is shown in figure 3. When $z=O$ equation (32) becomes $\frac{x^2}{a^2} + \frac{y^2}{b^2} = 1$, and the cross section through the plane $z=O$ (fig. 4) is an ellipse. When $y=O$, the equation becomes $\frac{x^2}{a^2} - \frac{z^2}{c^2} = 1$, and the section through the plane $y=O$ (fig. 5) is a hyperbola. Similarly, when $x=O$, $\frac{y^2}{b^2} - \frac{z^2}{c^2} = 1$ (fig. 6). Figure 7 indicates the two sets of straight-line generators which lie on the surface of an hyperboloid of one sheet.[2]

APPLICATION OF THE DEVELOPMENT TO A PROBLEM

In order to develop the equation which will describe the surface of a plow bottom, it is necessary to obtain the data called for in equations (16), (17), (18), and (19). This application of the development will be carried through for the bottom represented in Plate 6, A, which bottom was placed upon the machine shown in Plate 7, B, so that the origin of

[1] The constants a, b, and c of this equation do not necessarily have the same numerical values as in previous equations.

[2] The method for obtaining the equations of any line on the surface is given *in* SNYDER, Virgil, and SISAM, C. H. Op. cit., p. 93.

coordinates came at O, figure 8. The plane $y=O$ contains the points O, m, and n; and the plane $x=O$ contains the points O and m and is perpendicular to the plane $y=O$. The plane $z=O$ is perpendicular to both the planes $y=O$ and $x=O$. The axes are considered to be positive in the directions indicated by the arrowheads (fig. 8). Three transverse lines, ab, cd, and ef (fig. 8), were selected and the following data obtained:

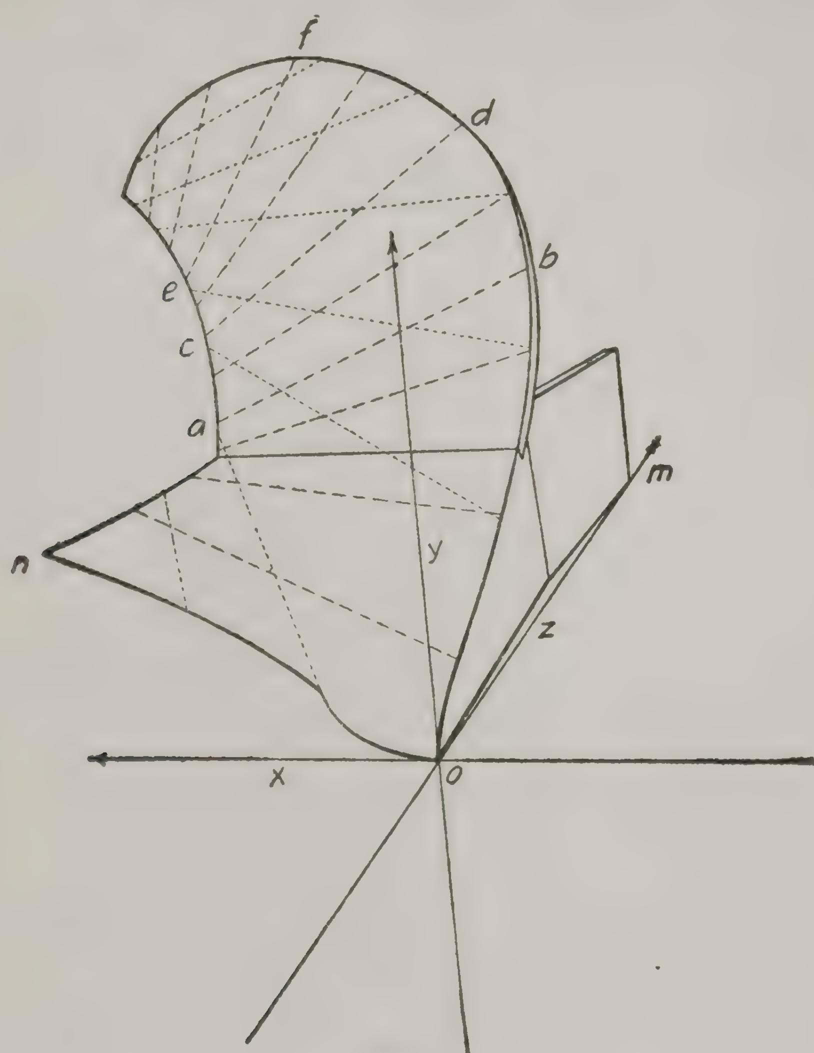


FIG. 8.

TABLE I.—*Values (in inches) developed for the surface of the plow bottom shown in Plate 6, A*

$x_1=2.84$	$x_2=7.42$	$x_3=4.42$
$y_1=5.7$	$y_2=3.78$	$y_3=8.74$
$z_1=16.0$	$z_2=19.0$	$z_3=20.0$
$x_4=8.54$	$x_5=9.7$	$x_6=12.58$
$y_4=6.43$	$y_5=10.88$	$y_6=7.65$
$z_4=23.0$	$z_5=26.0$	$z_6=28.0$

When the above values are substituted in the equations already developed,

From (16)

$$A=\frac{-2.28K+13.83}{K-15.7}; \tag{33}$$

From (17)

$$A = \frac{-x - 1.78y + 20}{1.299y + z - 31.35}. \tag{34}$$

From (33) and (34)

$$K = \frac{-15.7x + 10.04y - 13.83z + 11.95}{x - 1.177y - 2.28z + 51.45}. \tag{35}$$

From (18)

$$B = \frac{-1.584K + 6.335}{K - 7.29}; \tag{36}$$

From (19)

$$B = \frac{-x - .892y + 19.4}{.619y + z - 32.74}. \tag{37}$$

From (36) and (37)

$$K = \frac{7.29x + 2.58y - 6.335z + 65.85}{x + .088y - 1.54z + 32.46}. \tag{38}$$

By eliminating K from equations (35) and (38) the following equation for the surface of the plow bottom is obtained:

$$3.9x^2 + y^2 + 3.45z^2 - 7.53yz - 7.28xz + 6.79xy \\ + 87.1x + 120.75y - 75.05z + 227.25 = O. \tag{39}$$

Table II is compiled for purposes of checking the values computed from equation (39) with those obtained by measuring.

TABLE II.—*Values (in inches) for the surface of the plow bottom shown in Plate 6, A, obtained by measurement*

z	y	x	x computed from equation (39)	Difference.
10	2	2.9	2.27	0.63
15	6	1.53	1.56	−.03
15	4	3.58	3.77	−.19
15	2	6.9	6.32	.58
20	10	3.72	3.8	−.08
20	8	4.73	4.76	−.03
20	4	7.83	7.94	−.13
25	12	8.22	8.12	.1
25	9	9.07	9.2	−.13
25	6	10.43	10.46	−.03
30	10	14	13.86	.14
32	9	16.5	16.1	.4

To find the geometric center, substitute the coefficients from equation (39) into equations (22), (23), and (24). Solving, we find

$$x_0 = -1.405 \text{ inches.}$$
$$y_0 = 6.52 \text{ inches.}$$
$$z_0 = 16.4 \text{ inches.}$$

This translation of axes is shown in figure 9. From equation (25a) $G = -57.3$. From (25) the equation of the surface referred to parallel axes through the center is

$$3.9x^2 + y^2 + 3.45z^2 - 7.53yz - 7.28xz + 6.79xy - 57.3 = 0. \qquad (40)$$

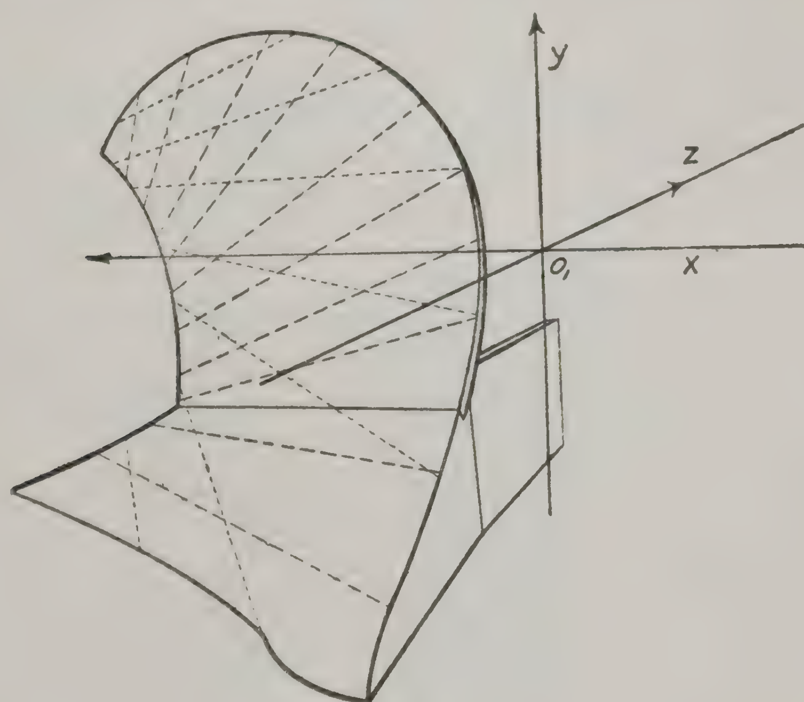


FIG. 9.

To find the equation of the surface referred to the principal axes through the center, substitute the coefficients from (39) into (26), and we have

$$k^3 - 8.35k^2 - 20.17k + 2.45 = 0. \qquad (41)$$

On solving by Horner's method

$$k_1 = 10.27$$
$$k_2 = 0.128$$
$$k_3 = -2.05$$

Substituting the values just found for k_1, k_2, k_3, D, and G in equation (27), we find

$$10.27x^2 + .128y^2 - 2.05z^2 = 57.3$$

or

$$\frac{x^2}{(2.36)^2} + \frac{y^2}{(21.2)^2} - \frac{z^2}{(5.29)^2} = 1. \qquad (42)$$

The direction cosines of the angles which the axes make after rotation with the original axes are obtained by making the proper substitutions in equations (28), (29), (30), and (31).

For the X-axis

$$\gamma = \mp 0.6136$$
$$\mu = \mp 0.48$$
$$\upsilon = \pm 0.627.$$

For the Y-axis

$$\gamma = \pm 0.7515$$
$$\mu = \mp 0.1437$$
$$\upsilon = \pm 0.6445.$$

For the Z-axis

$$\gamma = \mp 0.1415$$
$$\mu = \pm 0.828$$
$$\upsilon = \pm 0.5425.$$

Figure 10 shows the axes after translation and rotation and the portion of the hyperboloid of one sheet which is a close approximation to the surface of this plow bottom.

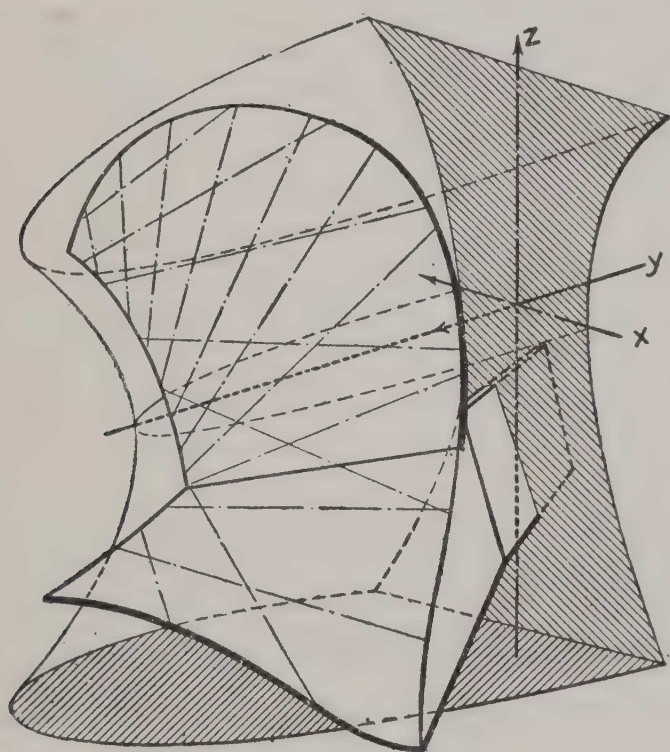


Fig. 10.

SURFACES ONE PORTION FROM EACH OF TWO QUADRIC SURFACES

By the use of the method which has just been employed to develop the equation of the surface of the plow bottom shown in Plate 6, A, two equations can be developed which will approximately represent the surface of the bottom shown in Plate 6, B. By taking the origin as at O, figure 8, the data of Tables III and IV were obtained from the share and the front portion of the moldboard.

Table III.—*Values (in inches) developed for the surface of the share and front portion of the moldboard of the plow bottom shown in Plate 6, B*

$x_1 = 3.92$	$x_2 = 7.4$	$x_3 = 1.73$
$y_1 = .8$	$y_2 = .75$	$y_3 = 2.67$
$z_1 = 8.0$	$z_2 = 12.0$	$z_3 = 12.0$
$x_4 = 6.78$	$x_5 = 2.36$	$x_6 = 5.87$
$y_4 = 1.75$	$y_5 = 4.05$	$y_6 = 2.7$
$z_4 = 16.0$	$z_5 = 15.0$	$z_6 = 17.0$

$$0.25x^2 + 2.34y^2 + 0.46z^2 - 3.25yz - 0.77xz + 2.66xy + 6.88x + 32.3y - 5.81z - 4.4 = O \qquad (43)$$

TABLE IV.—*Values (in inches) for the surface of the share and front part of the moldboard of the plow bottom shown in Plate 6, B, obtained by measuring*

z	y	x	x computed from equation (43)	Difference
10	1	4.75	4.75	0.00
10	2	1.75	1.54	.21
15	1	8.37	9.00	−.63
15	2	5.47	5.64	−.17
15	3	3.77	3.82	−.05
15	4	1.1	1.3	−.2

From the remaining surface of the moldboard the following data of Tables V and VI were obtained:

TABLE V.—*Values (in inches) of rest of surface of moldboard shown in Plate 6, B*

$x_1 = 8.67$ $x_2 = 4.96$ $x_3 = 11.73$
$y_1 = 4.95$ $y_2 = 8.64$ $y_3 = 4.81$
$z_1 = 24.0$ $z_2 = 22.0$ $z_3 = 29.0$

$x_4 = 9.08$ $x_5 = 13.62$ $x_6 = 12.24$
$y_4 = 9.0$ $y_5 = 6.23$ $y_6 = 11.89$
$z_4 = 27.0$ $z_5 = 33.0$ $z_6 = 31.0$

$$1.07x^2 - 1.07y^2 + z^2 - 3.99yz - 1.5xz + 16.37xy + 60.55x + 125.3y - 48.5z + 109.5 = O \qquad (44)$$

TABLE VI.—*Values (in inches) for the rest of the moldboard surface shown in Plate 6, B, obtained by measurement*

z	y	x	x computed from equation (44)	Difference.
20	2	8.85	8.68	0.17
20	4	6.67	6.78	−.11
20	6	4.9	4.95	−.05
20	8	3.4	3.5	−.1
25	3	10.6	10.5	.1
25	5	9.3	9.2	.1
25	7	8.23	8.12	.11
25	9	7.4	7.34	.06
25	11	6.82	6.77	.05
30	5	12.2	12.1	.1
30	7	11.7	11.63	.07
30	9	11.38	11.35	.03
30	11	11.3	11.24	.06
30	13	11.4	11.3	.1
35	5	14.65	14.53	.12
35	7	14.72	14.66	.06
35	9	15	14.93	.07
35	11	15.45	15.32	.13
35	13	16.1	15.85	.25
40	8	17.57	17.62	−.05
40	10	18.5	18.52	−.02

From a study of Tables II, IV, and VI it is evident that the share can not be as accurately described by mathematical equations as can the moldboard. However, the differences even upon the share are not very great. It must be remembered that these surfaces have been developed empirically; experience and an extensive knowledge of the conditions to be met have been the chief guides. Yet this implement produced in the school of experience has a surface approximately mathematically exact in form. Further, the surfaces of cast bottoms, which, because of the difficulty of manufacture, are not changed unless necessity demands, consist in some cases approximately of a portion from each of two quadric surfaces. It will be shown later in discussing the history of the plow that the surfaces of the Holbrook bottoms were designed to be portions of hyperboloids of one sheet. In the Utica (N. Y.) plow trials these machines received many first awards and much commendation from the judges for the excellence of their work. In addition to this, Mr. J. J. Washburn, of Batavia, N. Y., who knew Mr. Holbrook and was present at the Utica plow trials, stated that the Holbrook plows did as good work as any that it has ever been his pleasure to witness. Thus, there is considerable evidence, based upon field experience, which indicates that a portion of a hyperboloid of one sheet is the proper form for the surface of a plow bottom. So far as is known, this hypothesis awaits definite proof.

MOTION OF THE SOIL PARTICLES IN PLOWING

For the purpose of studying the motion of the soil particles in plowing, the work was limited to sod ground available in the vicinity of Ithaca, N. Y. From observations on a sod plow at work in the field (Pl. 7, C), the following general facts regarding the furrow slice were noted:

The lower outside [1] edge of the furrow slice did not appear to be either stretched or compressed.

The upper outside edge of the furrow slice appeared to be compressed.

The inside of the furrow slice was stretched, the lower edge more than the upper edge.

As the furrow slice passed over the moldboard the cracks, which had formed on the inside in traveling over the share and the front portion of the moldboard, closed up as the soil passed over the rear of the plow bottom, indicating a point of maximum stretching.

The above considerations made it evident that a more detailed study of the behavior of the furrow slice was desirable. For this purpose rows of pins were set in the unplowed ground, the pins being driven in the ground to the estimated depth of plowing, as shown in Plate 4, A. The longitudinal rows are parallel to the line of motion of the plow, which is also parallel to the Z-axis (fig. 8) and the transverse rows perpendicular

[1] The portion of the furrow slice immediately adjacent to the furrow is called the "outside."

to this same line of motion. The longitudinal rows are numbered from II to VI (Row I was omitted because the colter upset the pins), and the pins in each row numbered from 1 to 10, as shown in figure 11. When the part of the furrow slice in which the pins were set was upon the moldboard, it took the form shown in Plate 8, B. In order to obtain the *x*, *y*, and *z* coordinates of points in the furrow slice upon the moldboard, the apparatus shown in Plate 9, A, was used. In this apparatus the axes have the same relation to the plow bottom as those shown in figure 8. This more detailed study of the furrow slice upon the moldboard revealed the following:

The length of Row II, pins 1 to 10, on top of the furrow slice was greater than the length before the soil had passed upon the moldboard, indicating that this portion of the furrow slice had been stretched.

The length of Row II, pins 1 to 10, was greater upon the bottom of the furrow slice than its length before the soil passed upon the moldboard.

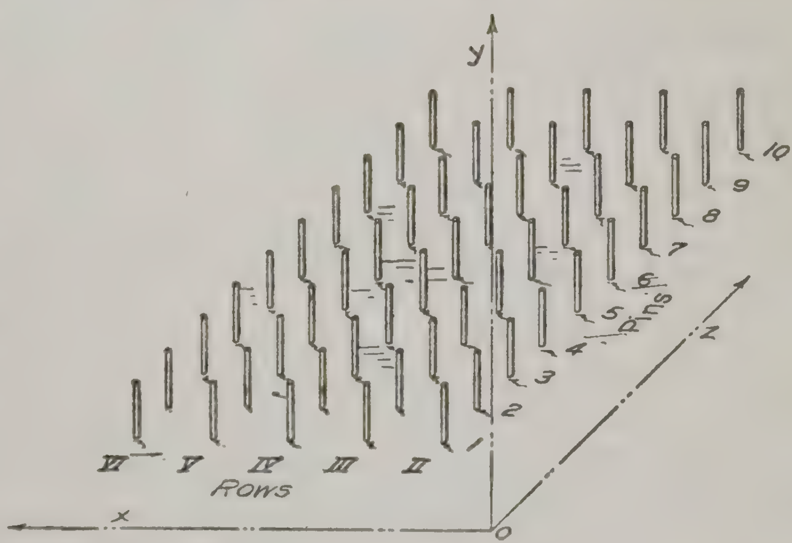


FIG. 11.

The length of Row VI, pins 1 to 10, on top of the furrow slice was less than its length before the soil passed upon the moldboard, indicating that this portion of the furrow slice had been compressed.

The length of Row VI, pins 1 to 10, on the bottom of the furrow slice was greater than its length before the soil passed upon the moldboard.

The lengths of Rows IV and V, pins 1 to 10, on top of the furrow slice were approximately the same as their lengths before the soil passed upon the plow bottom, indicating neither compression nor stretching.

The lengths of Rows IV and V, pins 1 to 10, on the bottom of the furrow slice was greater than their lengths before the soil had passed upon the plow bottom.

The *z* distances of pin 10 on top of the furrow slice were approximately the same for each row, but less than the distance which the plow had moved forward.

The *z* distances of pin 10 on the bottom of the furrow slice were approximately the same for each row and equal to the distance which the plow had moved forward. (The coordinates of the pins at the bottom of the furrow slice were measured by cutting away a portion of the soil but leaving the pins in place.)

These observations reveal, first, that when a cross section of the furrow slice is considered (fig. 12) the portion marked "A" is compressed in plowing and the portion marked "B" is stretched, while the soil in the position of line lj is neither compressed nor stretched; and, second, that there is a definite relation between the z coordinate of a soil particle and the distance the plow has moved forward. This relation is developed on pages 164 to 167.

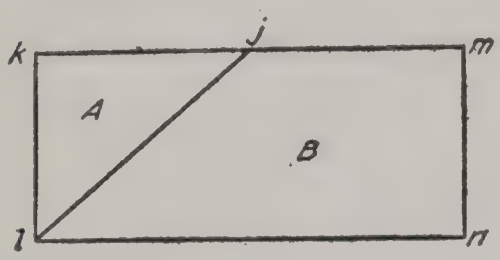


FIG. 12.

The next step was to analyze in detail the motion of the soil particles. This study was limited to the soil particles upon the bottom of the furrow slice, but the methods developed are applicable to other portions. The paths of the soil particles upon the bottom of the furrow slice can be very accurately traced from the scratches which they make upon the moldboard. Plate 9, B, shows the paths of five soil particles. By taking the axes as shown in figure 8, a projection of these paths upon the plane $z=O$ showed a very uniform set of curves. Each of these curves (fig. 13) can be very accurately described by equations of the general form

$$ax^2+by^2+lx+my+d=O. \tag{45}$$

When these same paths are projected upon the plane $y=O$, a set of curves resulted (fig. 14), each of which could be very accurately described by equations having the following general form:

$$ax^2+bz^2+lxz+mx+nz+d=O, \tag{46}$$

From equation (45) $\frac{dy}{dt}$ and $\frac{d^2y}{dt^2}$ the velocity and acceleration, respectively, of a soil particle in the y direction can be found if $\frac{dx}{dt}$ and $\frac{d^2x}{dt^2}$ are known. The values of $\frac{dx}{dt}$ and $\frac{d^2x}{dt^2}$ can be found from equation (46) if $\frac{dz}{dt}$ and $\frac{d^2z}{dt^2}$ are known. Thus, to analyse the velocity and acceleration of any soil particle whose path upon the surface of the plow bottom is known, an equation must be found between z and time (t).

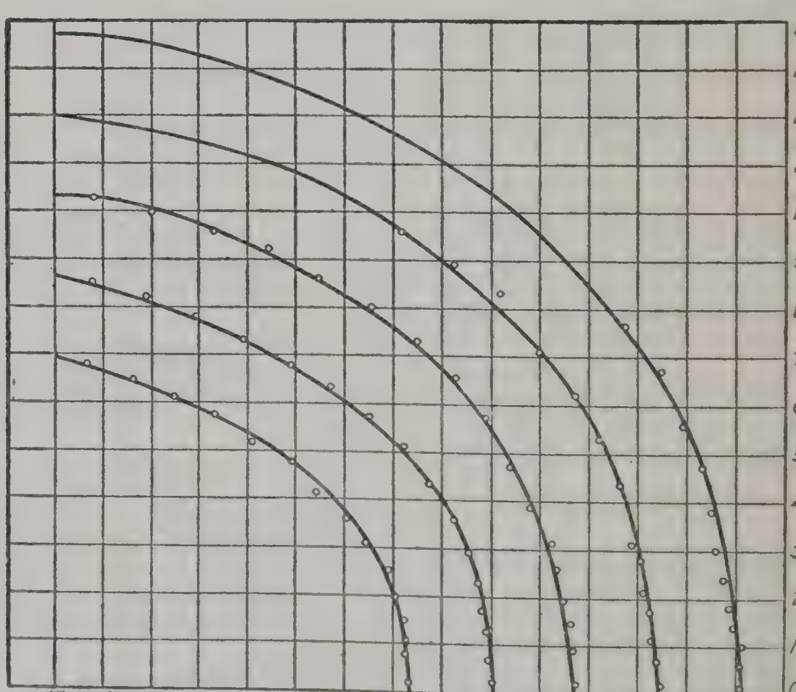


FIG. 13.—Projection of the paths shown in Plate 9, A, upon plane $z=O$.

This was accomplished by comparing the z coordinates of the bottom ends of the pins with the distance which the plow had moved forward. The distance which the plow moved forward is designated by s, so that

$$s = vt, \tag{47}$$

where v = velocity of the plow, and t = time.

By the use of the apparatus illustrated in Plate 9, A, the data given in Table VII were obtained for the soil particles upon the bottom of the furrow slice whose paths are shown in Plate 9, B. These data are typical of 12 sets of observations.

TABLE VII.—*Values (in inches) of points in the furrow slice*

Row II.			Row III.			Row IV.			Row V.		
z	s	$z-s$	z	s	$z-s$	z	s	$z-s$	z	s	$z-s$
$16\frac{1}{2}$	$15\frac{3}{4}$	$\frac{3}{4}$	16	$15\frac{3}{4}$	$\frac{1}{4}$	$15\frac{7}{8}$	$15\frac{3}{4}$	$\frac{1}{8}$	$15\frac{3}{4}$	$15\frac{3}{4}$	0
$20\frac{1}{2}$	$19\frac{3}{4}$	$\frac{3}{4}$	$20\frac{3}{8}$	$19\frac{3}{4}$	$\frac{5}{8}$	$19\frac{7}{8}$	$19\frac{3}{4}$	$\frac{1}{8}$	$20\frac{1}{4}$	$19\frac{3}{4}$	$\frac{1}{2}$
24	$23\frac{3}{4}$	$\frac{1}{4}$	$23\frac{7}{8}$	$23\frac{3}{4}$	$\frac{1}{8}$	$24\frac{1}{4}$	$23\frac{3}{4}$	$\frac{1}{2}$	$23\frac{3}{4}$	$23\frac{3}{4}$	0
$27\frac{3}{4}$	$27\frac{3}{4}$	0	$27\frac{5}{8}$	$27\frac{3}{4}$	$-\frac{1}{8}$	$27\frac{1}{2}$	$27\frac{3}{4}$	$-\frac{1}{4}$	$27\frac{1}{4}$	$27\frac{3}{4}$	$-\frac{1}{2}$
$32\frac{1}{4}$	$31\frac{3}{4}$	$\frac{1}{2}$	$32\frac{1}{8}$	$31\frac{3}{4}$	$\frac{3}{8}$	32	$31\frac{3}{4}$	$\frac{1}{4}$	$31\frac{7}{8}$	$31\frac{3}{4}$	$\frac{1}{8}$
$35\frac{5}{8}$	$35\frac{3}{4}$	$-\frac{1}{8}$	$35\frac{3}{4}$	$35\frac{3}{4}$	0	$35\frac{7}{8}$	$35\frac{3}{4}$	$\frac{1}{8}$	$35\frac{1}{4}$	$35\frac{3}{4}$	$-\frac{1}{2}$
$39\frac{3}{8}$	$39\frac{3}{4}$	$-\frac{3}{8}$	$39\frac{1}{2}$	$39\frac{3}{4}$	$-\frac{1}{4}$	$39\frac{5}{8}$	$39\frac{3}{4}$	$-\frac{1}{8}$	$39\frac{1}{4}$	$39\frac{3}{4}$	$-\frac{1}{2}$

Unfortunately the soil available in the vicinity of Ithaca was not well adapted for taking observations of the kind reported in Table VII. This soil is not uniform in texture, contains many stones, cracks much more readily than it stretches, and the surface is not as level as could be desired for this work. At times it was difficult to drive the pins straight into the ground. The data of Table VII show, however, a distinct tendency for the difference between z and s to reach a maximum value and then decrease again to zero; and also a slight tendency for this maximum difference to decrease from Row I to Row V. When the work was begun, it was hoped that sufficiently accurate data could be obtained from which a law between z and s could be developed, but on account of the difficulties already explained this was impossible. Consequently, in order to develop a method for future work, a set of conditions were assumed which agreed qualitatively with the observed facts. It should always be kept in mind that this was done simply as an hypothesis whose exactness should be thoroughly tested upon a soil better adapted to this work. The conditions assumed for the relations between z and s are as follows:

(A) That, for each path, when $z=40$, $s=40$.

(B) That there was no stretching or compression in the outside bottom edge of the furrow slice up to the point $z=40$.

(C) That the maximum difference, $z-s$, for Path I was 1.05 inches.

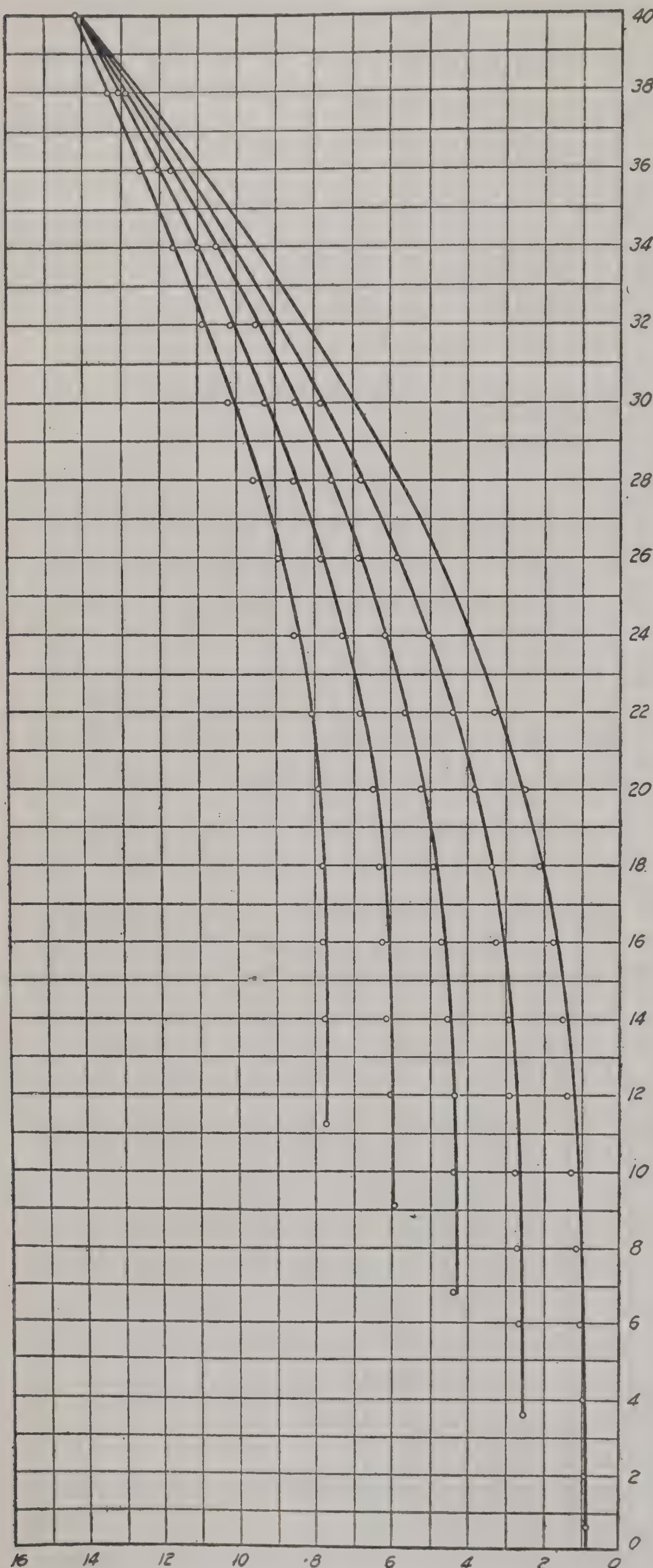


FIG. 14.—Projection of the paths shown in Plate 9, A, upon the plane $y=0$.

(D) That the maximum difference, $z-s$, for each path decreased uniformly across the furrow slice. Thus, for Row I, $x=0.85$ inch, the maximum $z-s=1.05$ inches, and when $x=13.6$ inches, the width of the furrow-slice, the maximum $z-s=0$; so when $x=7.5$ inches, the maximum $z-s$ for Row V is 0.45 inch.

(E) That the stretching in each row took place uniformly up to the maximum point and then decreased uniformly until it was zero when $z=s=40$.

(F) That the maximum stretching occurred midway between the point where the soil particle passed upon the plow bottom and the point $s=40$. Thus, for Path I where the soil particle passed upon the moldboard at the point $s=0.6$:

$$40 - 0.6 = 39.4 \text{ inches.}$$
$$39.4 \div 2 = 19.7 \text{ inches.}$$
$$19.7 + 0.6 = 20.3 \text{ inches.}$$

For Path I the point of maximum stretching was at $s=20.3$ inches.

The computations below show that for Path V, where the soil

particle passed upon the share at the point $s=11.6$, the point of maximum stretching occurs at $s=25.8$ inches.

$$40 - 11.6 = 28.4 \text{ inches.}$$
$$28.4 \div 2 = 14.2 \text{ inches.}$$
$$14.2 + 11.6 = 25.8 \text{ inches.}$$

The following is the simplest form of a function which meets the requirements imposed by the above conditions and, when the constants are determined, will describe the relations between z and s for a soil particle on the bottom of the furrow slice as it passes over the surface of the plow bottom:

$$z-s=a(s^2+bs+c)^2 \tag{48}$$

From equations (47) and (48)

$$z-vt=a[(vt)^2+bvt+c]^2; \tag{49}$$

From (49) $\frac{dz}{dt}$ and $\frac{d^2z}{dt^2}$, the velocity and acceleration, respectively, of a soil particle in the z direction can be obtained.

From equation (46) by differentiation we have

$$(2ax+lz+m)\frac{dx}{dt}+(2bz+lx+n)\frac{dz}{dt}=O; \tag{50}$$

and

$$(2ax+lz+m)\frac{d^2x}{dt^2}+\left(2a\frac{dx}{dt}+l\frac{dz}{dt}\right)\frac{dx}{dt}$$
$$+(2bz+lx+n)\frac{d^2z}{dt^2}+\left(2b\frac{dz}{dt}+l\frac{dx}{dt}\right)\frac{dz}{dt}=O. \tag{51}$$

Similarly from equation (45) we find

$$(2ax+l)\frac{dx}{dt}+(2by+m)\frac{dy}{dt}=O; \tag{52}$$

and

$$(2ax+l)\frac{d^2x}{dt^2}+2a\left(\frac{dx}{dt}\right)^2+(2by+m)\frac{d^2y}{dt^2}+2b\left(\frac{dy}{dt}\right)^2=O. \tag{53}$$

From equations (50), (51), (52), and (53) the velocities $\frac{dx}{dt}$, $\frac{dy}{dt}$, and the accelerations $\frac{d^2x}{dt^2}$, $\frac{d^2y}{dt^2}$ of a soil particle on the bottom of the furrow slice can be obtained when $\frac{dz}{dt}$ and $\frac{d^2z}{dt^2}$ are known.

In this problem, however, we are interested in the accelerations in the directions of the normal to the surface, designated by "*N*," the tangent to the soil path "*T*," and the perpendicular to the plane formed by the normal and the tangent "*R*."

We can find λ_1, μ_1, v_1, the direction cosines of the angles which N makes with the X-, Y-, and Z-axis in either of the following ways:

If (20) (the equation of the surface of the plow bottom) is known, we have by differentiation

$$\frac{\lambda_1}{ax_0+by_0+gz_0+l}=\frac{\mu_1}{hx_0+by_0+fz_0+m}$$

$$=\frac{v_1}{gx_0+fy_0+cz_0+n}= \tag{54}$$

$$\frac{1}{\sqrt{\begin{matrix}(ax_0+by_0+gz_0+l)^2+(hx_0+by_0+fz_0+m)^2\\+(gx_0+fy_0+cz_0+n)^2\end{matrix}}};$$

or if the paths of the soil particles are known but the equation of the surface is unknown the angle Ny can be measured by means of a protractor and plumb bob, as shown in Plate 9, C. The direction cosines λ_1 and v_1 can then be computed from the following:

$$(\lambda_1)^2+(\mu_1)^2+(v_1)^2=1 \tag{55}$$

$$\lambda_1\frac{dx}{dt}+\mu_1\frac{dy}{dt}+v_1\frac{dz}{dt}=O; \tag{56}$$

where the values for $\frac{dx}{dt}$, $\frac{dy}{dt}$, and $\frac{dz}{dt}$ can be obtained from (49), (50), and (52).

The direction cosines of T (λ_2, μ_2, v_2) are proportional to $\frac{dx}{dt}$, $\frac{dy}{dt}$, and $\frac{dz}{dt}$. Hence

$$\frac{\lambda_2}{\frac{dx}{dt}}=\frac{\mu_2}{\frac{dy}{dt}}=\frac{v_2}{\frac{dz}{dt}}=\frac{1}{\sqrt{\left(\frac{dx}{dt}\right)^2+\left(\frac{dy}{dt}\right)^2+\left(\frac{dz}{dt}\right)^2}} \tag{57}$$

The direction cosines of T (λ_3, μ_3, v_3) can be computed from the following:[1]

$$(\lambda_3)^2+(\mu_3)^2+(v_3)^2=1. \tag{58}$$

$$\frac{\lambda_1}{\mu_3 v_2-v_3\mu_2}=\frac{\lambda_2}{\mu_3 v_1-v_3\mu_1}=\frac{\lambda_3}{\mu_1 v_2-v_1\mu_2}=\pm 1. \tag{59}$$

The components in the directions N, T, and R of the forces acting on a soil element of mass M, moving with the component accelerations $\frac{d^2x}{dt^2}$, $\frac{d^2y}{dt^2}$ and $\frac{d^2z}{dt^2}$ are

$$F_N=M(\lambda_1\frac{d^2x}{dt^2}+\mu_1\frac{d^2y}{dt^2}+v_1\frac{d^2z}{dt^2}) \tag{60}$$

$$F_T=M(\lambda_2\frac{d^2x}{dt^2}+\mu_2\frac{d^2y}{dt^2}+v_2\frac{d^2z}{dt^2}) \tag{61}$$

$$F_R=M(\lambda_3\frac{d^2x}{dt^2}+\mu_3\frac{d^2y}{dt^2}+v_3\frac{d^2z}{dt^2}). \tag{62}$$

[1] SNYDER, Virgil, and SISAM, C. H. Op. cit., p. 40.

EVALUATING THE CONSTANTS IN EQUATIONS (48), (46), AND (45)

The methods of evaluating the constants in equations (48), (46), and (45) for a given soil path will now be considered. For this purpose Path V (Pl. 9, A) will be taken. The general form of equation (48) is

$$z-s=a(s^2+bs+c)^2. \qquad (48)$$

From the assumptions that have already been made (p. 164 to 168) the following data for this curve are obtained:

s	z
11.6	11.6
25.8	26.25
40.0	40.0

On substituting the above values for s and z in equation (48), three equations are obtained from which it is found that

$$a=0.00001114$$
$$b=-51.6$$
$$c=464$$

giving

$$z-s=0.00001114(s^2-51.6s+464)^2 \qquad (63)$$

To determine the values of the constants in

$$ax^2+bz^2+lxz+mx+nz+d=O, \qquad (46)$$

the origin is moved to $x=7.65$, $z=11.6$. For this point as origin an equation of the following form describes the curve:

$$a(x')^2+b(z')^2+l_1x'z'+m_1x'=O. \qquad (64)$$

Taking $a=1$, only three constants, b, l_1, and m_1, remain to be evaluated. From the trace of Path V on the surface of the plow bottom the following data were obtained:

x'	z'
1	13.55
3	20.05
6	27.15

Substituting these values for x' and z' in equation (64) gives three equations from which

$$b=-0.019$$
$$l_1=-0.453$$
$$m_1=\ \ 8.63$$

$$(x')^2-0.019(z')^2-0.453x'z'+8.63x'=O. \qquad (65)$$

Translating the axes back to the original origin,

$$x'=x-\ \ 7.65$$
$$z'=z-11.6$$

gives

$$x^2-0.019z^2-0.453xz-1.45x+3.91z-49.92=O. \qquad (66)$$

To determine the values of the constants in

$$ax^2+by^2+lx+my+d=O, \tag{45}$$

the origin is moved to, $x=7.65$, $y=0.2$. This changes the form of the equation to

$$a(x')^2+b(y')^2+l_1x'+m_1y'=O. \tag{67}$$

Taking $a=1$, three constants remain to be evaluated. From the trace of Path V upon the surface of the plow bottom,

x'	y'
1	3.1
4	5.45
7	6.68

Substituting these values of x' and y' in equation (67) gives

$$\begin{aligned} a&=\ 1 \\ b&=\ 4.29 \\ l_1&=-30.85 \\ m_1&=-\ 3.67 \end{aligned}$$

$$(x')^2+4.29(y')^2-30.85x'-3.67y'=O. \tag{68}$$

The axes are translated back to the original origin by substituting

$$\begin{aligned} x&=x'-7.65 \\ y&=y'-0.2 \end{aligned}$$

in equation (68), which gives

$$x^2+4.29y^2-46.15x-5.39y+295.45=O. \tag{69}$$

NUMERICAL EXAMPLE

The surface of a plow bottom is represented by the equation

$$\begin{aligned} 0.54x^2-1.52y^2+1.12z^2-3.69yz-1.62xz+2.04xy \\ +53.63x+114.90y-46.4z+49.4=O. \end{aligned}$$

The motion of a soil particle which passes upon this bottom at the point $x=6.9$, $y=0.2$, $z=9.5$ is described by the following equations:

$$z=0.00001622(s^2-45.5s+342)^2+s \tag{70}$$

$$-0.119z^2-1.126xz+20.78x+10.03z-201.63=O \tag{71}$$

$$x^2+1.8y^2-42.41x-1.5y+245.25=O \tag{72}$$

$$s=vt. \tag{47}$$

From equations (70), (71), (72), and (47) the following are obtained:

TABLE VIII.—*Values (in inches) for*—

s	z	x	y
18	18.4	7.55	3.6
27	27.4	11.5	8.25
36	36.0	19.5	11.0

$$z=0.00001622(v^2t^2-45.5vt+342)^2+vt \tag{73}$$

$$\frac{dz}{dt}=0.00003244[(v^2t^2-45.5vt+342)(2v^2t-45.5v)]+v \tag{74}$$

$$\frac{d^2z}{dt^2}=0.00003244[(v^2t^2-45.5vt+342)(2v^2)+(2v^2t-45.5v)^2] \tag{75}$$

$$\frac{dx}{dt}=\frac{(.238z+1.126x-10.03)\frac{dz}{dt}}{2x-1.126z+20.78} \tag{76}$$

$$\frac{d^2x}{dt^2}=\frac{(0.238z+1.126x-10.03)\frac{d^2z}{dt^2}-2\left(\frac{dx}{dt}\right)^2+0.238\left(\frac{dz}{dt}\right)^2+2.25^2\left(\frac{dx}{dt}\right)\left(\frac{dz}{dt}\right)}{2x-1.126z+20.78} \tag{77}$$

$$\frac{dy}{dt}=\frac{(-2x+42.41)\frac{dx}{dt}}{3.6y-1.5} \tag{78}$$

$$\frac{d^2y}{dt^2}=\frac{(-2x+42.41)\frac{d^2x}{dt^2}-2\left(\frac{dx}{dt}\right)^2-3.6\left(\frac{dy}{dt}\right)^2}{3.6y-1.5} \tag{79}$$

The plow moved forward with a velocity of 36 inches per second, giving

$$s=36t \tag{80}$$

From equations (74), (75), (76), (77), (78), (79), and (80) the values listed in Table IX are computed.

TABLE IX.—*Values for*—

s	t	$\frac{dx}{dt}$	$\frac{d^2x}{dt^2}$	$\frac{dy}{dt}$	$\frac{d^2y}{dt^2}$	$\frac{dz}{dt}$	$\frac{d^2z}{dt^2}$
	Sec.						
18	½	7.09	53.6	16.9	28.4	37.7	−9.07
27	¾	25.15	47.75	17.32	−50.0	34.44	−10.21
36	1	38.4	41.6	3.44	−74.8	36	29.52

By making the proper substitutions from (80), (74), (76), and (78) in equations (54), (57), (58), and (59) the values of the direction cosines for the normals N the tangents to the path T, and the perpendiculars to the planes formed by the normals and tangents R for three points are computed and listed in Table X.

TABLE X.—*Values of the direction cosines for normals, tangents to the path, and perpendiculars to the planes*

$x=7.55$ $y=3.6$ $z=18.4$

$\cos N_x=$ 0. 549 $\cos N_y=$. 716 $\cos N_z=-$. 429	$\cos T_x=$ 0. 169 $\cos T_y=$. 4025 $\cos T_z=$. 9	$\cos R_x=$ 0. 817 $\cos R_y=-$. 564 $\cos R_z=$. 0977

$x=11.5$ $y=8.25$ $z=27.4$

$\cos N_x=$ 0. 728 $\cos N_y=$. 229 $\cos N_z=-$. 646	$\cos T_x=$0. 546 $\cos T_y=$. 376 $\cos T_z=$. 749	$\cos R_x=$ 0. 4145 $\cos R_y=-$. 897 $\cos R_z=$. 149

$x=19.5$ $y=11$ $z=36$

$\cos N_x=$ 0. 698 $\cos N_y=-$. 215 $\cos N_z=-$. 683	$\cos T_x=$0. 728 $\cos T_y=$. 065 $\cos T_z=$. 683	$\cos R_x=$ 0. 102 $\cos R_y=-$. 975 $\cos R_z=$. 2

For the purpose of computing the forces a block of soil 2 inches wide, 1 inch long, and ½ inch thick is taken. The mass of this soil is

$$M=\frac{(2.1.5)62.5\rho}{1728.32.2.12}=\frac{0.0362\rho}{32.2.12} \tag{81}$$

$\rho=$density.

By the proper substitutions from Tables IX and X into equations (60), (61), and (62) the forces necessary to produce the accelerations are computed and listed in Table XI.

TABLE XI.—*Forces necessary to produce acceleration in soil particles*

$x=7.55$	$y=3.6$	$z=18.4$
$F_N=$ .00503ρ	$F_T=$ .00116ρ	$F_R=$ .00252ρ
$x=11.5$	$y=8.25$	$z=27.9$
$F_N=$ .00281ρ	$F_T=$ .000248ρ	$F_R=$ .00592ρ
$x=19.5$	$y=11$	$z=36$
$F_N=$ .00234ρ	$F_T=$ .00428ρ	$F_R=$ .00778ρ

A soil particle in passing over the surface of the plow bottom will be acted upon by the following:

(*a*) A force from the surface of the bottom acting in the direction of the normal.

(*b*) Gravity.

(*c*) Pressure from the weight of the soil above the particle.

(*d*) Friction between the particle and the surface.

(*e*) Shearing, stretching, or compression on each of the remaining five sides of the particle, due to its contact with other soil particles.

The force which produces the movement of a soil particle in any direction will be the resultant of the components of the above-listed forces which act in the direction of the movement.

The preceding analysis of the motion which certain soil particles have in the operation of plowing has not been developed from as refined methods nor as uniform data in all cases as could be desired, but the results obtained furnish abundant evidence that the problem here attempted is by no means hopeless. The study should be continued upon a tough sod, which would stretch more uniformly, and some apparatus which would remove the necessity of certain soil particles remaining in line with each other should be substituted for the pins.

HISTORY OF THE DEVELOPMENT OF PLOW BOTTOMS

The Annual Report of the New York State Agricultural Society for 1867 contains an excellent treatise giving the geometrical construction of the surfaces of many historical plow bottoms, but no attempt has been made in that report to classify these surfaces upon the basis of their mathematical forms. Using the above-mentioned work as a basis, the author has attempted to work out the mathematical forms of the most important of these historical surfaces with a view to making fundamental comparisons with present-day plow bottoms.

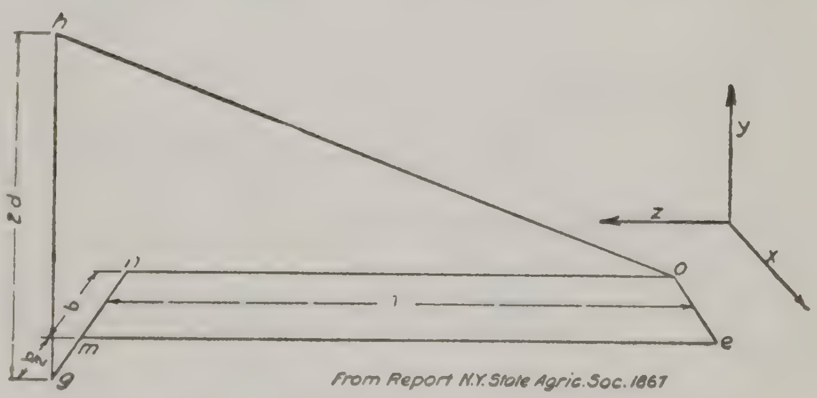


FIG. 15.

JEFFERSON'S PLOW BOTTOM

In 1788 Thomas Jefferson, while making a tour in Germany, developed what appears to be one of the first methods recorded for making the surface of the moldboard geometrically exact in form.[1] He argued that the offices of the moldboard were to receive the soil from the share and invert it with the least possible resistance. In order to do this, Jefferson developed a surface which he considered best adapted for the work of plowing, but attention should be called to the fact that no evidence is offered to prove the assertion. Figure 15 shows the framework for generating the Jefferson moldboard, in which lines *em* and *oh* are the directrices. To generate the surface a straightedge is laid upon *eo* and

[1] GOULD, J. S., et al. Op. cit., p. 403.

moved backward, the straightedge remaining parallel to the plane $z=O$. By taking the point o as the origin, the equation of the surface is

$$3byz-2dxz-2bly+2bdz=O^{1} \tag{82}$$

b = breadth of furrow
d = depth of furrow
l = length of moldboard.

On rotating the XY-axes through $\tan^{-1}=2d/3b$, the equation is

$$(9b^2+4d^2)y'z-4bdlx'-6b^2ly'+2bd\sqrt{9b^2+4d^2}z=O. \tag{83}$$

On rotating the $Y'Z$-axes through $\tan^{-1}\frac{1}{2}\sqrt{2}$, the equation is $(9b^2+4d^2)$ $[(y'')^2-(z')^2]-8bdlx'$

$$+2(bd\sqrt{18b^2+8d^2}-3b^2l\sqrt{2})[y''+z']=O. \tag{84}$$

Translating the axes to the points

$$y''=y'''+y_0$$
$$z'=z''+z_0$$

where y_0 has such a value that

$$2(9b^2+4d^2)y_0+2[bd\sqrt{18b^2+8d^2}-3b^2l\sqrt{2}]=O, \tag{85}$$

and z_0 has such a value that

$$-2(9b^2+4d^2)z_0+2[bd\sqrt{18b^2+8d^2}-3b^2l\sqrt{2}]=O, \tag{86}$$

gives

$$(9b^2+4d^2)[(y''')^2-(z'')^2]-8bdlx'+(y_0^2-z_0^2)(9b^2+4d^2)$$
$$+(y_0+z_0)(2bd\sqrt{18b^2+8d^2}-3b^2l\sqrt{2})=O. \tag{87}$$

Letting the constant terms in (87) equal C gives

$$(9b^2+4d^2)[(y''')^2-(z'')^2]-8bdlx'+C=O. \tag{88}$$

Translating the axes to the point $x'=x''+x_0$ where x_0 has such a value that

$$-8bdlx_0+C=O$$

gives

$$(9b^2+4d^2)[(y''')^2-(z'')^2]=8bdlx''. \tag{89}$$

This is the equation of a hyperbolic paraboloid.[2]

LAMBRUSCHINI'S PLOW BOTTOM

Lambruschini,[3] an Italian, describes a method for generating the surface of a plow bottom which he considered to be more efficient than the surface developed by the Jefferson method. Lambruschini proposed

[1] The method of developing the equation for this surface is given upon pages 150 to 156.
[2] SNYDER, Virgil, and SISAM, C. H. Op. cit., p. 73.
[3] LAMBRUSCHINI, R. Op. cit., p. 37–80. 1832.

a helacoid generated as follows: Lay out a rectangle *opan* (fig. 16) twice the desired width of the furrow and of an empirically determined length. Take the point m midway between points o and p and draw the line mm_1 parallel to pq. A straightedge laid upon mo and moved backward along the line mm_1 being kept parallel to the plane $z=O$, and with an angular rotation proportional to the movement toward m_1, generates the surface of the Lambruschini bottom. The point of the straightedge which was at o will describe the helix oo_1q (fig. 16). The equation of this surface is

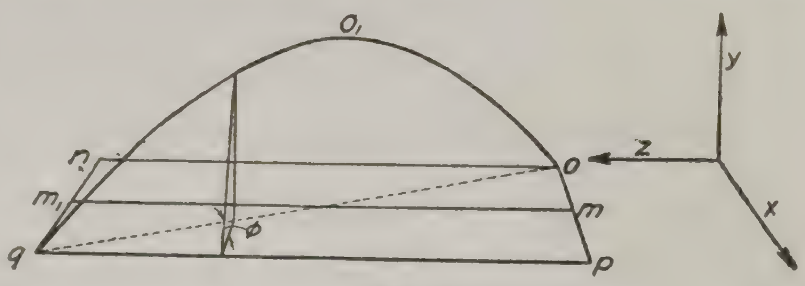


FIG. 16.

$$\frac{y}{x}=\tan\theta,$$

where θ has uniformly increasing values as z increases.

Then $\theta=f\ (z)$, when $\theta=90°=\frac{n}{2}$ radians,

$$z=\frac{l}{2},$$

$l=$length of line mm_1

$$\frac{n}{2}=c\frac{l}{2}$$

$$c=\frac{n}{l}.$$

Hence,
$$\frac{y}{x}=\tan\left(\frac{n}{l}z\right). \tag{90}$$

SMALL'S PLOW BOTTOM [1]

About 1760, a Scotchman, James Small, established a factory in Scotland for the manufacture of plows. The surface of Small's moldboard is obtained by laying a straightedge upon op (fig. 17) and moving it backward parallel to the plane $z=O$, with the line pm and the curve oh as directrices. The equation of the curve, a half catenary, is obtained by drawing a line og (fig. 18) the length of line og (fig. 17). At o erect a line oo_1 perpendicular to line og and equal in length to line gh (fig. 17).

FIG. 17.

[1] GOULD, J. S., et al. Op. cit., p. 415.

Through point o_1 (fig. 18) draw a line o_1h parallel and equal to line og. With h and o as points of suspension describe a catenary with its lowest point at O. Taking the point O (fig. 18) as origin, the equation of the catenary is

$$y=\frac{a}{2}(e^{2lz/3ba}+e^{-2lz/3ba}). \tag{91}$$

$$a=Og.$$

Transferring the origin to the point o gives

$$y=\frac{a}{2}(e^{2lz/3ba}+e^{-2lz/3ba})-a \tag{92}$$

as the equation of the catenary oh (fig. 17). The equations of line pm (fig. 17) are

$$y=O$$
$$x=b.$$

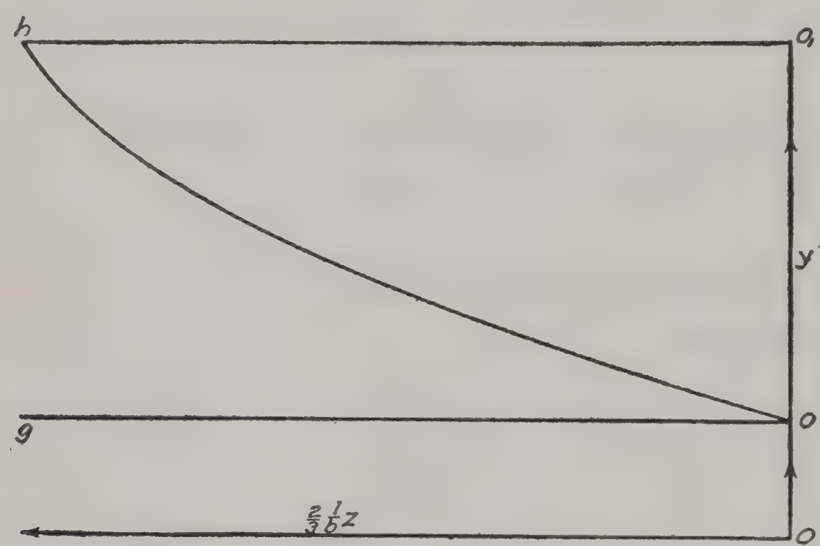


FIG. 18.

Any plane parallel to the plane $z=O$ is given by $z=c$, and this plane cuts the line pm at the point

$$x_1=b$$
$$y_1=O$$
$$z_1=c.$$

It also cuts the catenary oh at the point

$$x_2=\frac{3b}{2l}c$$
$$y_2=f(c)$$
$$z_2=c.$$

The equation of the line in the plane $z=c$ which cuts the line pm and the catenary oh (fig. 17) is

$$\frac{x-b}{\frac{3b}{2l}c-b}=\frac{y-O}{f(c)-O} \tag{93}$$

or

$$(x-b)f(c)-y\left(\frac{3b}{2l}c-b\right)=O \tag{94}$$

As this line is always parallel to the plane $z=O$, it follows that $c=z$ and

$$f(c)=f(z).$$

From equations (92) and (94) then,

$$(x-b)\left[\frac{a}{2}\left(e^{2lz/3ba}+e^{-2lz/3ba}\right)-a\right]-y\left(\frac{2b}{3l}c-b\right)=O, \qquad (95)$$

which is the equation of Small's moldboard.

STEPHEN'S PLOW BOTTOM [1]

About the same time that Small brought out his moldboard another Scotchman named Stephens developed a method for forming the surface

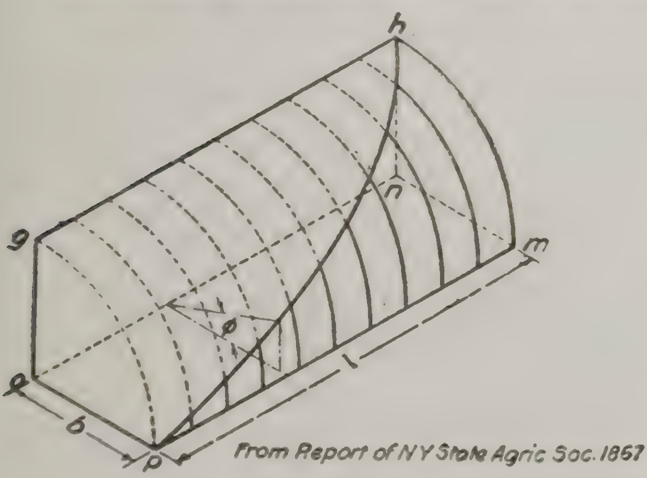


FIG. 19.

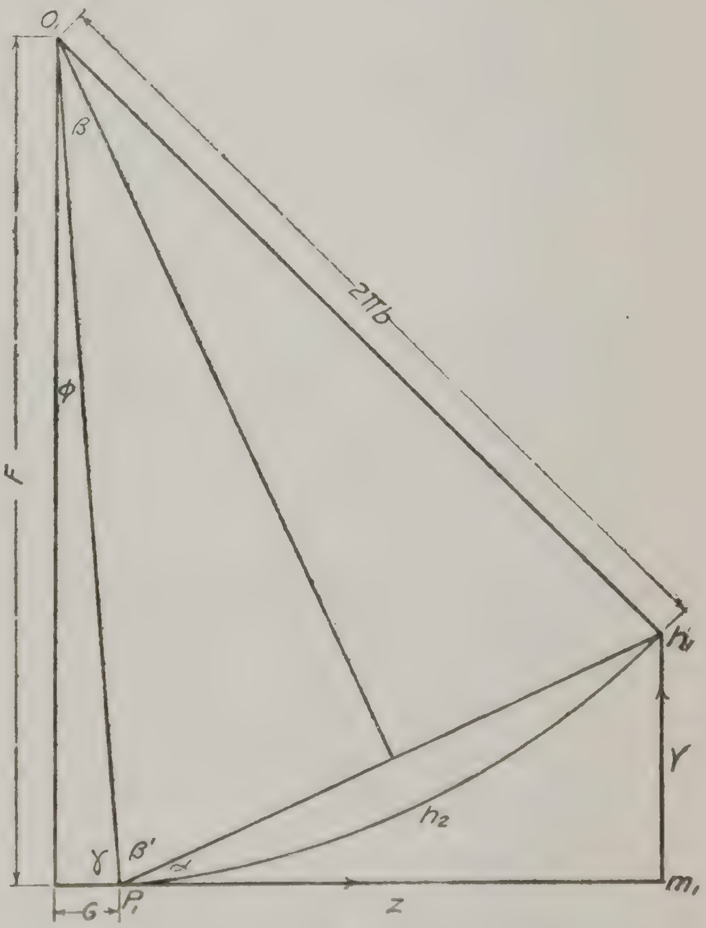


FIG. 20.

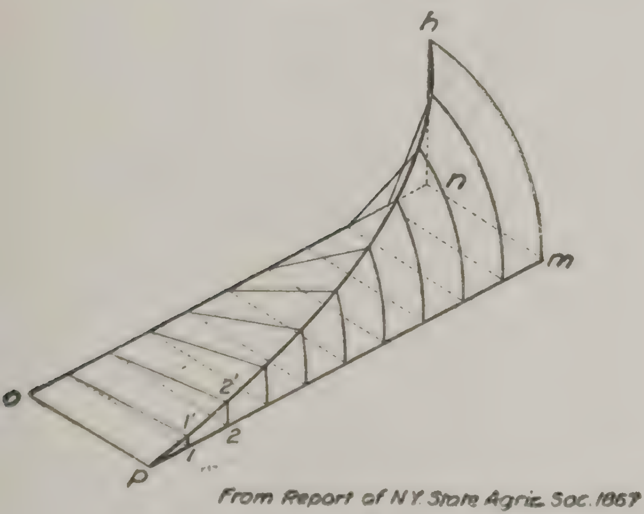


FIG. 21.

of a moldboard the general plan of which is shown in figure 19. The generator for this surface is a straightedge laid upon *op* (fig. 19) and moves backward parallel to the plane $z=O$ with the line *on* and the curve *ph* as directrices. Stephen designed his surface by taking a quarter cylinder *opmnhg* and laying out p_1m_1 (fig. 20) equal in length to *pm* (fig. 19). Perpendicular to line p_1m_1 draw m_1h_1 equal to the length of arc *mh* (fig. 19). Through points p, h_1 (fig. 20) pass a circle of radius $2\pi b$. The plane figure $p_1m_1h_1h_2$ (fig. 20) is then laid upon the quarter cylinder (fig. 19) so that p_1 falls upon p, m_1 upon m, and h_1 upon h. This will locate the curve *ph* (fig. 19), leaving a figure as shown in figure 21. It will be

[1] GOULD, J. S., et al. Op. cit., p. 431.

observed in figure 21 that $\frac{y}{x}=\tan\theta$ where θ has gradually increased values from O at $z=O$ to 90° at $z=l$. Further, $\theta=\frac{\gamma}{b}$, radians where γ represents the lengths of arcs 11′, 22′, etc.; then $\frac{y}{x}=\tan\left(\frac{\gamma}{b}\right)$. From figure 20 the equation of the circle with its center at O, taking p_1 as the origin is

$$(\gamma-F)^2+(z+G)^2=4n^2b^2 \tag{96}$$

$$\gamma=F+\sqrt{4n^2b^2-(z-G)^2} \tag{97}$$

In figure 20

$$\begin{aligned}\phi+\ \gamma&=\ 90°;\\ B+B'&=\ 90°;\\ a+B'+\ \gamma&=180°;\\ \phi&=a-B;\end{aligned}$$

$$F=2nb\cos\phi$$

$$=\frac{l\sqrt{4n^2b^2-\left(\frac{l^2}{4}+\frac{n^2b^2}{16}\right)}}{2\sqrt{l^2+\frac{n^2b^2}{4}}}+\frac{nb}{4}; \tag{98}$$

$$G=2nb\sin\phi$$

$$=\frac{nb\sqrt{4n^2b^2-\left(\frac{l^2}{4}+\frac{n^2b^2}{16}\right)}}{2\sqrt{l+\frac{n^2b^2}{4}}}-\frac{l}{2nb}. \tag{99}$$

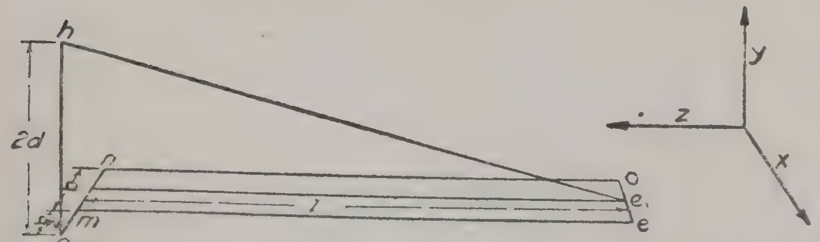
Substituting the values for F from equation (98) and for G from equation (99) gives

$$\frac{y}{x}=\tan\,[f(z)], \tag{100}$$

which is the equation of the surface.

RAHM'S PLOW BOTTOM [1]

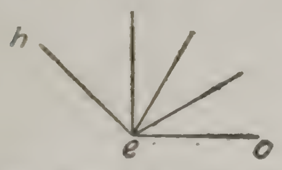
In 1846 Rev. W. L. Rham, an Englishman, brought forward the theory that the lines of the moldboard running in the longitudinal direction should be straight, but that the section of the moldboard formed by any plane $z=c$ (fig. 22) should be a straight line or a curve, according to the physical characteristics of the soil to be worked. Mr. Rham agreed that for medium, mellow soils the surface of the moldboard should be



From Report of N. Y. State Agric. Soc. 1867.
FIG. 22.

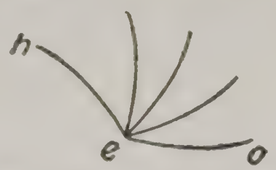
[1] GOULD, J. S., et al. Op. cit., p. 442.

generated by laying a straightedge upon *oe* and moving it backward parallel to the plane $z=O$ with the lines e_1h and *em* as directrices. This surface will be a portion of a hyperbolic paraboloid, the same general type as the surface which Jefferson proposed. The orthogonal projection of the generator in various positions upon the plane $z=O$ will look as shown in figure 23. For stiff, clay soils the lines (fig. 24)



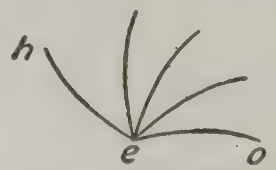
From Report of N. Y. State Agric. Soc. 1867

Fig. 23.



From Report of N. Y. State Agric. Soc. 1867

Fig. 24.



From Report of N. Y. State Agric. Soc. 1867

Fig. 25.

are made concave and for loose, sandy soils (fig. 25) they are made convex. As no exact description was given regarding the shape of the curves (fig. 24, 25), it has not been possible to develop equations for the surfaces. However, as it is known that these surfaces have straight lines in one direction and can not be described by an equation of the second order, they are of the fourth order or higher.

KNOX'S PLOW BOTTOM [1]

In 1852 Samuel A. Knox, of Worcester, Mass., applied for a patent upon the surface of a plow bottom which was certainly unique. The skeleton of this surface is shown in figure 26. The segments of circles I, II, and III are placed in parallel planes 12 inches apart, so that a series of straight lines will cut the three circles. Circles I and III have equal diameters and the diameter of circle II is one-half that of circles I and III. As the equation of this surface is of the eighth order, it will not be worked out in detail, but a development will be given to show how the equation could be obtained.

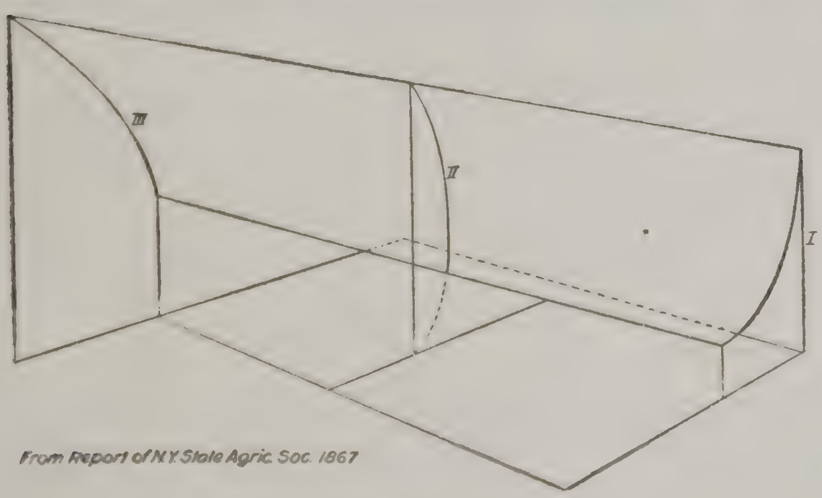


Fig. 26.

Let the equation of the three circles be [2]

$$x^2+y^2=R^2$$
$$z=O,$$
$$(x-a)^2+(y-b)^2=\left(\frac{R}{2}\right)^2$$
$$z=k;$$

[1] Gould, J. S., et al. Op. cit., p. 495.

[2] This development is the work of Virgil Snyder, Professor of Mathematics, Cornell University.

and

$$(x-c)^2+(y-d)^2=R^2$$
$$z=2k.$$

Draw the line from a point (x_1, y_1, O) on the first circle to a point $(x_2, y_2, 2k)$ on the third. Its equations are

$$\frac{x-x_1}{x_2-x_1}=\frac{y-y_1}{y_2-y_1}=\frac{z}{2k},$$

from which

$$\frac{2k(x-x_1)+z(x_1-c)}{z}=x_2-c,$$

$$\frac{2k(y-y_1)+z(y_1-d)}{z}=y_2-d.$$

Since

$$(x_2-c)^2+(y_2-d)^2=R^2,$$

we have, after simplifying,

$$4k^2[(x-x_1)^2+(y-y_1)^2]+4kz[(x-x_1)(x_1-c)$$
$$+(y-y_1)(y_1-d)]+z^2[(x_1-c)^2+(y_1-d)^2-R^2]=O. \qquad (101)$$

This is the equation of a cone with vertex at (x_1, y_1, O) and passing through the third circle.

In the same way, find the equations of the line from $(x_1, y_1\ O,)$ to (x_3, y_3, k) on the middle circle

$$\frac{x-x_1}{x_3-x_1}=\frac{y-y_1}{y_3-y_1}=\frac{z}{k},$$

$$\frac{k(x-x_1)+z(x_1-a)}{z}=x_3-a,$$

$$\frac{k(y-y_1)+z(y_1-b)}{z}=y_3-b.$$

Since

$$(x_3-a)^2+(y_3-b)^2=\left(\frac{R}{2}\right)^2,$$

we have, after simplifying,

$$k^2[(x-x_1)^2+(y-y_1)^2]+2kz[(x-x_1)(x_1-a)$$
$$+(y_1-b)(y-y_1)]+z^2\left[(x_1-a)^2+(y_1-b)^2-\frac{R^2}{4}\right]=O. \qquad (102)$$

When equations (101) and (102) are multiplied out, it will be seen that $x^2{}_1$, $y^2{}_1$ always enter in the form $x^2{}_1+y^2{}_1=R^2$. By substituting R^2 for $x^2{}_1+y^2{}_1$ in each, the equations are of the form

$$Ax_1+By_1=C,$$
$$A'x_1+B'y_1=C'.$$

Solve these equations for x_1, y_1 and put their values in

$$x^2_1+y^2_1=R^2.$$

$$A=[4kz(x+c)-2cz^2-8xk^2],$$
$$B=[4kz(y+d)-2dz^2-8yk^2],$$
$$C=[4R^2k^2-4k^2(x^2+y^2)-4kxz-4kzy-4kR^2z+z^2(c^2+d^2)]$$
$$A'=[2kz(x+a)-2xk^2-2az^2],$$
$$B'=[2kz(y+b)-2yk^2-2bz^2],$$
$$C'=[R^2k^2+k^2(x^2+y^2)-4kz(ax+by-R^2)+z^2(a^2+b^2+\frac{3}{4}R^2)].$$
$$x_1=\frac{B'C-BC'}{AB'-A'B},$$
$$y_1=\frac{C'A-CA'}{AB'-A'B},$$

hence
$$(B'C-BC')^2+(C'A-CA')^2=R^2(AB'-A'B)^2. \qquad (103)$$

CYLINDRICAL PLOW BOTTOMS

In 1854 an American, Joshua Gibbs,[1] patented a plow bottom the surface of which is a portion of a circular cylinder. Taking a point upon the axis of the cylinder as the origin, the equation of this surface is

$$\frac{x^2}{r^2}+\frac{y^2}{r^2}-1=O \qquad (104)$$

In some foreign countries, notably Germany, the hyperbolic cylinder has been suggested as suitable for forming the surface of the moldboard. In this connection it is interesting to note that any cylindrical surface can be described by an equation of the general form.

$$\frac{x^2}{a^2}\pm\frac{y^2}{b^2}\pm 1=O \qquad (105)^2$$

MEAD'S PLOW BOTTOM [3]

In 1863 a Mr. Mead, of New Haven, Conn., patented a plow bottom, the surface of which conformed exactly to a portion of a frustrum of a cone. The general equation of this surface is

$$\frac{x^2}{a^2}+\frac{y^2}{b^2}-\frac{z^2}{c^2}=O \qquad (106)$$

HOLBROOK'S PLOW BOTTOM

The Report of the New York State Agricultural Society for 1867 contains a very complete report of the plow trials held at Utica, N. Y., in 1867, at which trials a line of plows designed by F. F. Holbrook, of Boston, Mass., showed general superiority to all other makes. The

[1] Gould, J. S., et al. Op. cit., p. 502.
[2] Snyder, Virgil, and Sisam, C. H. Op. cit., p. 82.
[3] Gould, J. S., et al. Op. cit., p. 505.

following quotation gives a very good description of the Holbrook surfaces:

We [1] were interested in the most minute details of these plows by Gov. Holbrook and the trials at Utica and subsequently at Brattleboro, Vt., showed very clearly the influence of the warped surface which is generated by his method upon the texture of the soil. Gov. Holbrook is as yet unprotected by a patent on his method, and we are therefore most reluctantly compelled to withhold a description of it but we have no hesitation in saying that it is the best system for generating the true curve of the moldboard which has been brought to our knowledge. This method is applicable to the most diversified forms of plows, to long or short, to broad or narrow, to high or low, no matter what the form may be, this method will impress a family likeness upon them all. There will be straight lines in each running from the front to the rear and from the sole to the upper parts of the share and moldboard. None of these lines will be parallel to each other, nor will any of them be radii from a common center. The angle formed by any two of them will be unlike the angle formed by any other two; a change in the angle formed by any transverse lines will produce a corresponding change in the vertical lines, and there will always, in every form of this plow, be a reciprocal relation between the transverse and vertical [2] lines. Plows made upon this plan may appear to the eye to be as widely different as it is possible to make them, and yet, on the application of the straightedge and protractor, it will be found that they agree precisely in their fundamental character. The surface of the moldboard is always such that the different parts of the furrow slice will move over it with unequal velocities.

From the above description it is evident that the surfaces of the Holbrook plows are portions of a hyperboloid of one sheet whose general equation is

$$\frac{x^2}{a^2}+\frac{y^2}{b^2}-\frac{z^2}{c^2}=1$$

MISCELLANEOUS PLOW BOTTOMS

In addition to the surfaces already described there remain at least three which show unique characteristics, but data were not available for developing the equations.

In 1818 Gideon Davis,[3] of Maryland, patented the surface of a plow bottom which was obtained by using the segment of a circle as a generator and two segments of another circle as directrices. Somewhat later, 1834, James Jacobs,[4] another American, brought out a plow bottom the surface of which was a combination of two mathematical surfaces, each of which had sets of straight lines in two directions.

In 1839 Samuel Witherow, of Gettysburg, Pa., and David Pierce, of Philadelphia, Pa., brought out a plow bottom whose surface was generated by the most ingenious use of the arc of a cycloid. A more detailed description of this plow can be found in the Report of the New York State Agricultural Society for 1867.[5]

[1] GOULD, J. S., et al. Op. cit., p. 586.

[2] It should be noted that the lines here called transverse are designated as longitudinal (Pl. 2, A), and the lines called vertical are designated as transverse.

[3] GOULD, J. S., et al. Op. cit., p. 452.

[4] Idem, p. 486.

[5] Idem, p. 491.

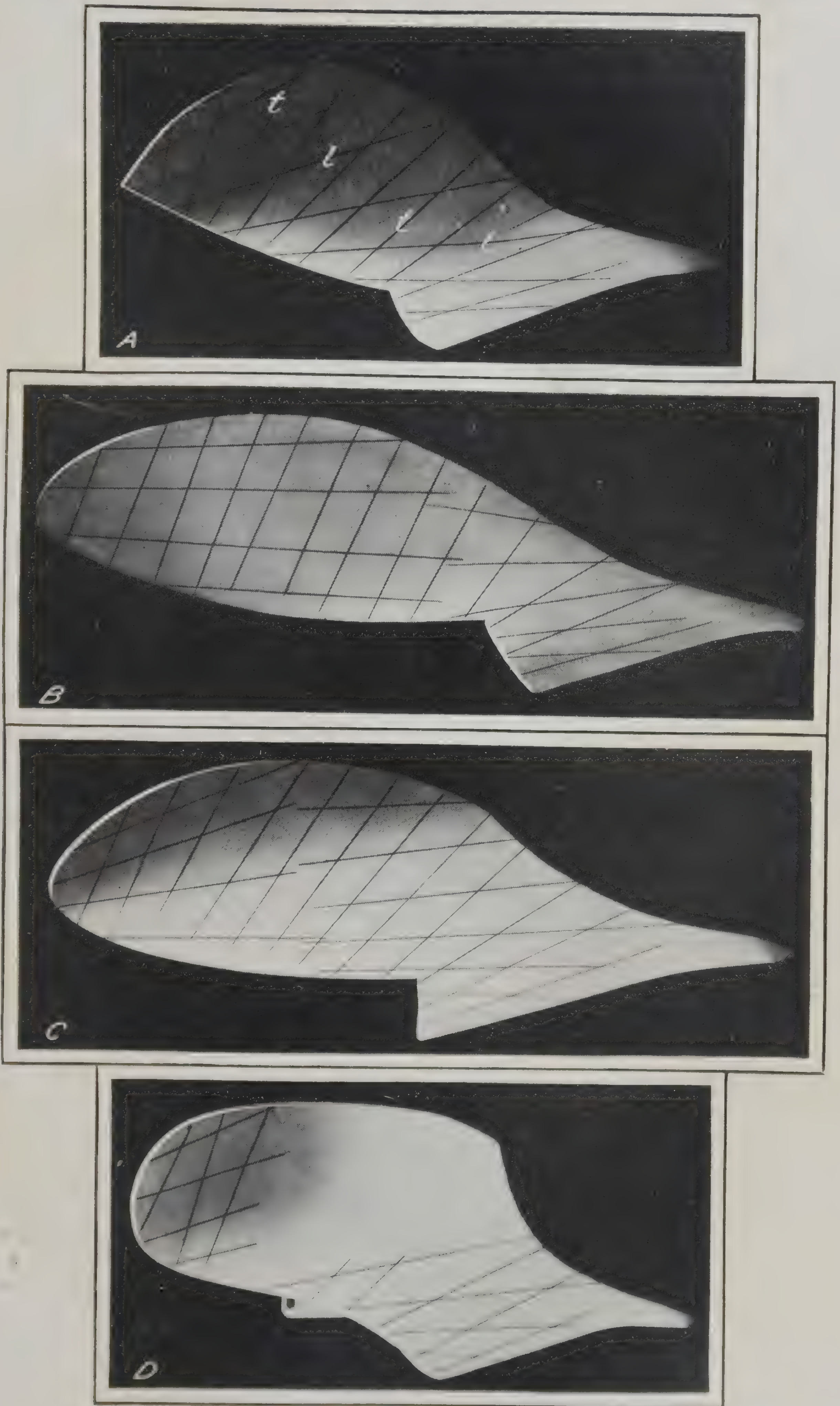
PLATE 6

A.—A plow bottom with two sets of straight lines.

B.—A plow bottom, the surface of which is composed of each of two surfaces.

C.—A plow bottom similar to B, but with the surfaces merging into each other farther back on the moldboard.

D.—A plow bottom, the surface of which does not contain an infinite set of straight lines.


A
B
C
D

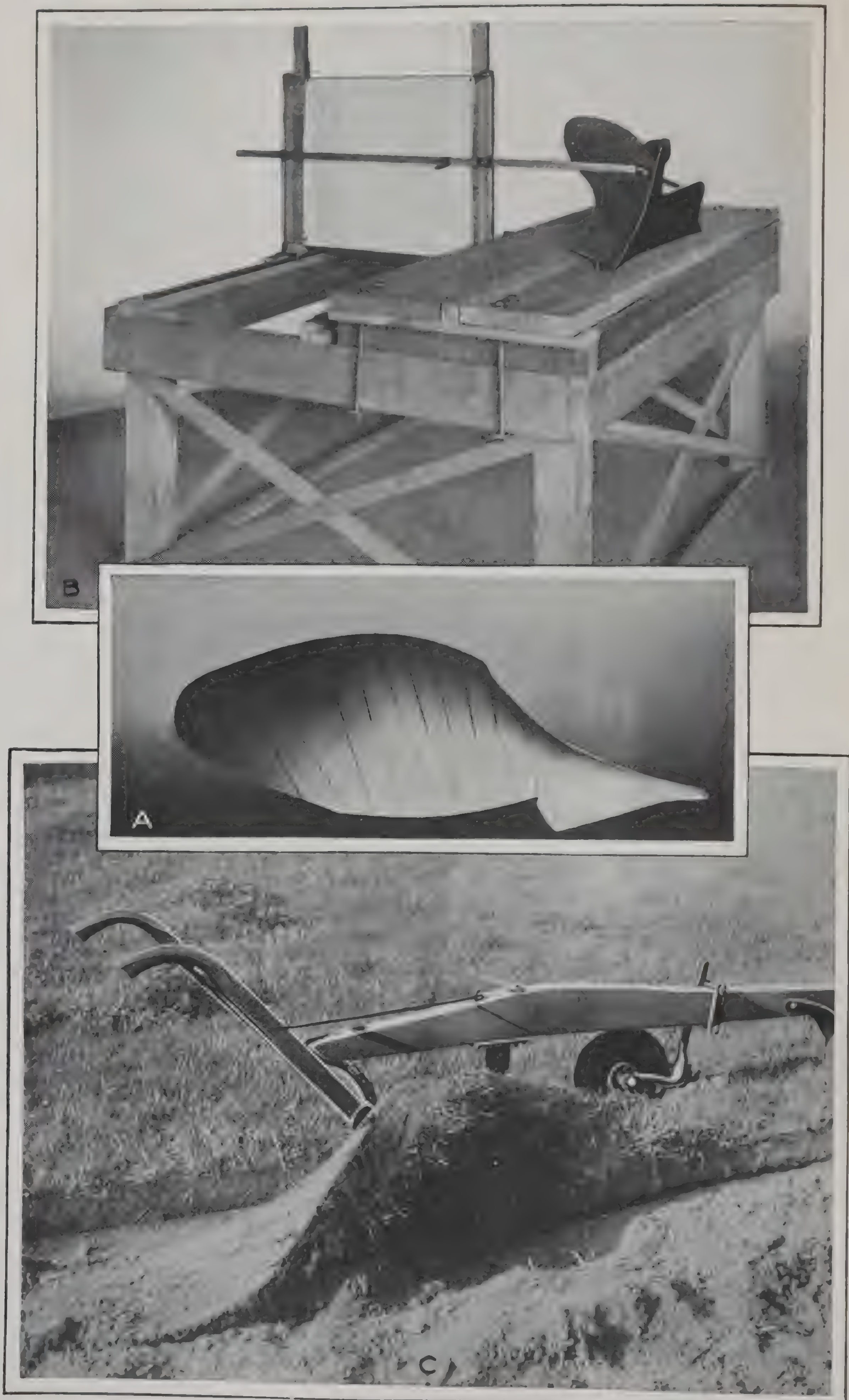

B
A
C

PLATE 7

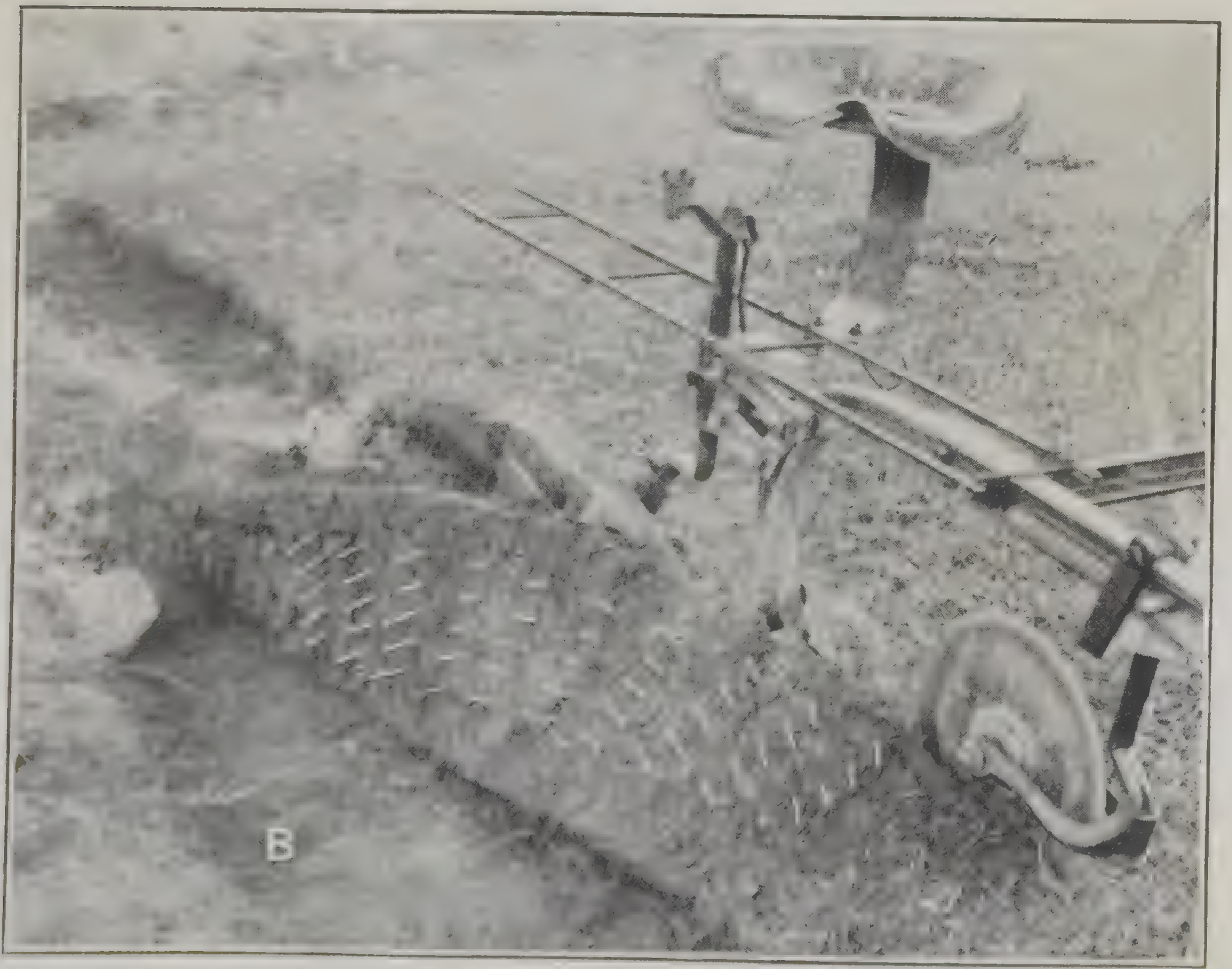
A.—A plow bottom with a convex surface which has two sets of straight lines.
B.—Instrument for measuring the space coordinates of any point of the plow bottom.
C.—A sod plow showing the furrow slice turned by it.

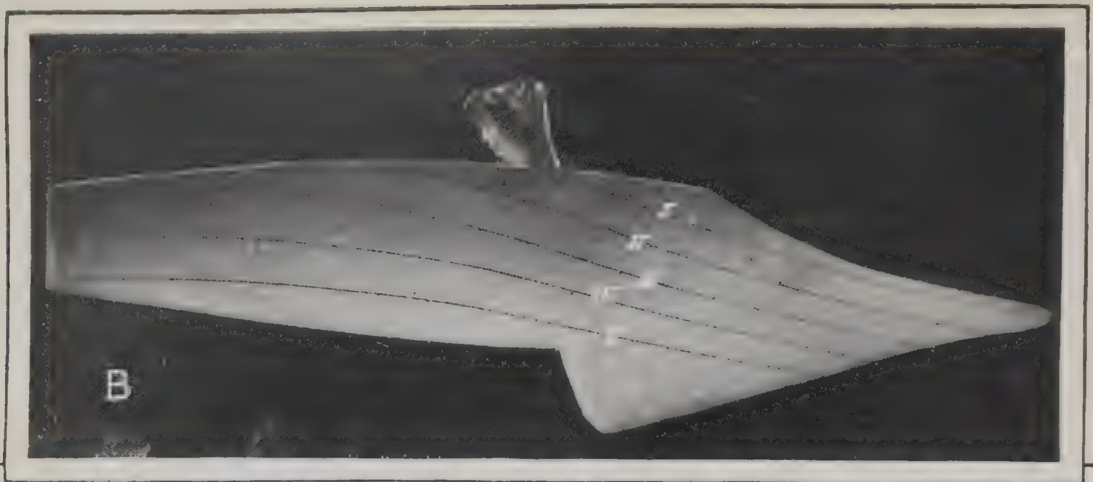
PLATE 8

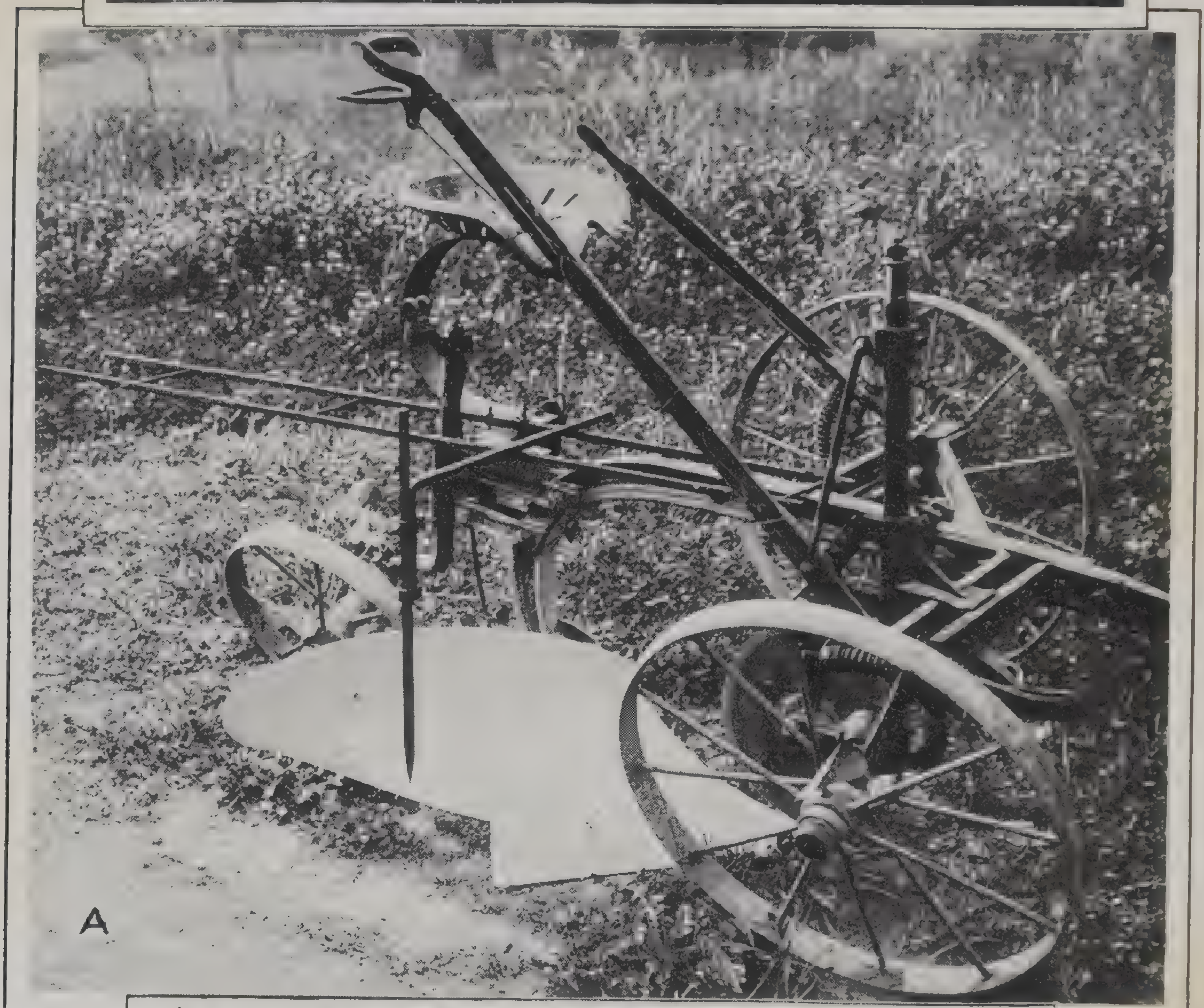
A.—Rows of wooden pins driven into the sod for estimating the stretch of the furrow slice.

B.—Furrow slice showing the position of the pins when on the moldboard.


A


B


B


A

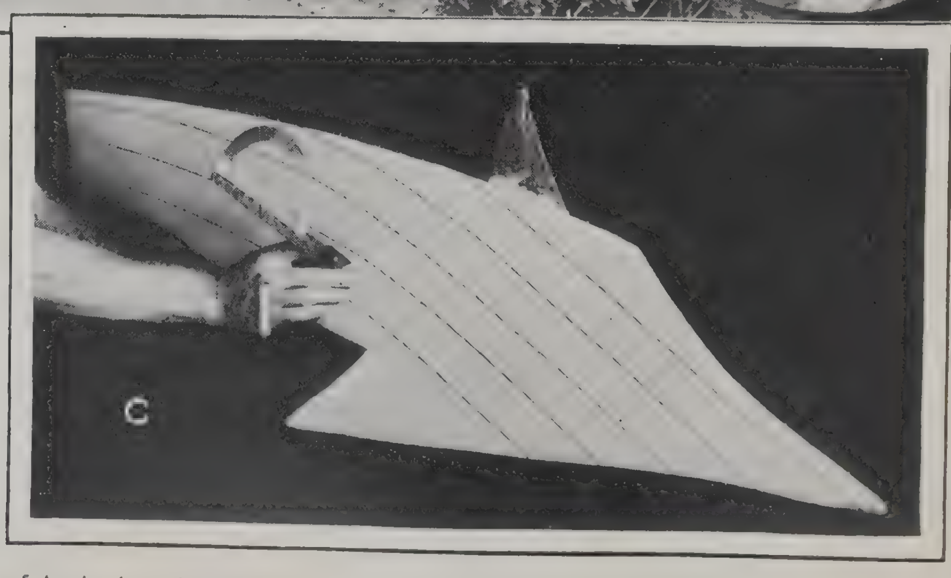

C

PLATE 9

A.—Plow showing attachment used to obtain the x, y, and z coordinates of points in the furrow slice.

B.—Moldboard showing the paths of five soil particles.

C.—Measurement of the angle Ny by use of a protractor and a plumb bob.

INFLUENCE OF NITRATES ON NITROGEN-ASSIMILATING BACTERIA[1]

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INTRODUCTION

RELATION OF NITRATES TO VARIOUS FORMS OF PLANT LIFE

The importance of nitrogen to plant life can not be overestimated. It is one of several elements essential to plant growth, one, moreover, which is apt to be deficient in arable soils. These facts are well brought out by the almost innumerable investigations which have been made concerning the source of nitrogen for plants.

The influence of nitrate nitrogen on various plants has been the controlling idea in many of these experiments. Very little attention has been placed on the effect of nitrates on the lower plants, especially the bacteria. Because of the relation that exists between higher plants and bacteria it seems advisable to consider the effect of nitrates on the soil bacteria. Indeed, progress in the knowledge of nitrogenous fertilizers depends on a study of the effect of the fertilizer on the soil organisms as well as on the higher plants. The action of fertilizers on the different groups of soil organisms, the relation of these organisms to higher plants, and the separation of the important from the unimportant groups are some of the factors involved in the problem of soil fertility.

REVIEW OF LITERATURE

The relation of nitrates to the germination of seeds has been studied by De Chalmot (*11*)[3], who found that corn germinated in solutions containing nitrate was more robust than corn germinated under similiar conditions without nitrate. He also noted that if too concentrated solutions of nitrate were used germination was retarded rather than hastened. The presence of nitrate also increased the amount of albuminous material in the seed.

The direct influence of nitrate nitrogen on the growing plant is too well known to justify any lengthy discussion here. Jost (*26, p. 134*) gives the results of experiments made by Boussingault, who grew the sunflower (*Helianthus argophyllus*) in sand with and without nitrate.

[1] Major portion of a paper submitted in partial fulfillment of the requirements for the degree of doctor of philosophy in bacteriology in the Graduate School of the University of Wisconsin, December, 1916.

[2] The writer wishes to acknowledge his appreciation of the suggestions and criticisms obtained throughout the progress of this work from Prof. E. B. Fred and E. G. Hastings, of the University of Wisconsin.

[3] Reference is made by number (italic) to "Literature cited," pp. 227–230.

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During the three months' growth of the plants 1.40 gm. of potassium nitrate were added. At the end of the period the dry weight of the plant supplied with nitrate was nearly 60 times greater than that of the plant where no nitrate was added. The relation between the growth of nonleguminous plants and the amount of nitrate nitrogen supplied is shown in a very striking manner in the following table taken from Hellriegel and Wilfarth (*21, p. 53-54*):

Nitrogen as $Ca(NO_3)_2$ added to pots, gm.	None	0.056	0.112	0.168	0.224	0.336
Dry weight of oats (grain and straw) gm.	0.3605 .4191	5.9024 5.8510 5.2867	10.9814 10.9413	15.9974	21.2732 21.4409	30.1750

But little work has been done on the direct influence of nitrates on the development of the Eumycetes. Some investigations have been made as to the ability of certain fungi to assimilate nitrate nitrogen directly. Ritter (*42*) studied many species and found that some forms would assimilate nitrate directly, while others reduced it to nitrite and ammonia. He found some forms which failed to grow on media containing nitrate. Kossowicz (*28*) found that various fungi utilized nitrates and that nitrite and ammonia were produced.

Münter (*36*) studied the influence of inorganic salts on the growth of various Actinomycetes. He found that potassium and sodium nitrates in quantities equivalent to 5 per cent permitted good growth of the organisms but retarded spore formation. Calcium, barium, and strontium nitrates in small quantities affected some species but not others. Small quantities of these nitrates did not affect growth to any extent, but larger quantities were detrimental to growth and spore formation. Silver nitrate in all amounts studied almost entirely prohibited growth.

Nitrates appear to exert some influence on the yeasts. Drabble and Scott (*13*) studied the effect of sodium nitrate on these organisms. They found that the greatest reproduction took place in solutions containing 0.2 gram-molecule of the nitrate. Increasing amounts of the salt led to a decrease in reproductive activity until with 0.7 gram-molecule present no reproduction took place. From their results it is evident that small quantities of nitrate stimulated reproduction, whereas larger amounts proved detrimental. Kayser (*27*) studied the effect of manganese nitrate on yeasts. He found that the amount which produced the maximum increase in the alcoholic fermentation of sugar varied with the strain of yeast employed. He likewise found that manganese nitrate produced greater increase than did the same quantity of potassium nitrate. Fernbach and Lanzenberg (*14*) concluded that nitrates hindered the rapidity of cell multiplication of yeasts but greatly accelerated the action of the zymase. More alcohol was formed in the presence than in the absence of nitrate. According to Kossowicz (*28*), nitrates are not a suitable source of nitrogen for yeasts.

The direct influence of nitrates on bacteria has been studied to a limited extent. The influence of various nitrates on soil bacteria has been studied by Greaves (*19*). He added sodium, potassium, calcium, magnesium, manganous and ferric nitrates to soil in varying quantities. The amount added to the soil was such that in each case equivalent quantities of the anion (NO_3) in the various forms were added. The effect of these salts on the bacteria was determined by using ammonification as an index of the bacterial activity. He found that sodium-potassium, manganous and ferric nitrates in small amounts, approximately 0.97 to 5.5 mgm. of nitrate in 100 gm. of soil, slightly stimulated ammonification. Greater concentrations of these salts proved toxic as evidenced by a decrease in the amount of ammonia formed. Sodium nitrate was much more beneficial to ammonification than potassium nitrate. From his results as a whole Greaves concludes that it is the electronegative ion which stimulates bacterial activity. Calcium and magnesium nitrates proved toxic in all concentrations studied.

However, a majority of the investigations have been directed toward a determination of the effect of the bacteria on the nitrates. But little work appears to have been done on the direct action of nitrates on bacteria. Pfeffer (*38, p. 351*) cites some experiments showing the repellant action of potassium nitrate toward certain bacteria. *Spirillum undula* was repelled by a solution of potassium nitrate having an osmotic concentration equivalent to 0.5 to 1.0 per cent. With *Spirillum volutans* a much higher concentration was necessary to bring about the same reaction. It was found that different organisms required different quantities of the same nitrate to repel them.

It can be readily seen that by far the greatest amount of work on the relation of nitrates to plant growth has been done in the realm of the higher plants. Obviously further investigations should be made in respect to the effect of nitrates on the lower forms of plant life, especially the bacteria. In this paper an attempt is made to set forth the results secured in a study of the influence which nitrates exert on certain groups of soil bacteria, including not only their reproduction but also some of their physiological properties.

EXPERIMENTAL WORK

OUTLINE OF PROBLEM

The results of much careful experimentation show that nitrate nitrogen is most readily assimilated by higher plants. As a rule it seems to stimulate the plant to increased activity. In some cases this is undoubtedly due to increased nutrition, while in others it is a result of nuclear stimulation with a consequent cell multiplication. No sharp line can be drawn between these two effects. Probably one overlaps the other, and the increased growth of the organism can be attributed to a combination of the two actions.

From a practical standpoint the relation of nitrates to the nitrogen-assimilating organisms of the soil is of importance. Hence, it was arranged to study the effect of nitrates on soil bacteria, especially those forms concerned with the fixation of atmospheric nitrogen. The work naturally falls into two rather distinct lines of investigation. First, the influence of nitrates on Azotobacter was determined. Here studies were made of the effect of nitrates on the growth of the organism in soil and also the effect of these salts on the nitrogen-fixing property of these bacteria. The action of Azotobacter on nitrates in solution, the relation of nitrates to pigment production and to the formation of volutin bodies were studied. Second, the influence of nitrates on the growth of *Bacillus radicicola* in soil was studied. The action of *B. radicicola* on nitrates in solution and the possible nitrogen-assimilating properties of the legume in the presence of nitrates were investigated. Also the influence of nitrates on gum production was determined. The latter part of the investigations included a study of the relation of nitrates to nodule formation on alfalfa.

METHODS USED IN EXPERIMENTS

Nitrates were determined by the reduction method with Devarda's alloy and also by the phenolsulphonic acid (colorimetric) method.

The total nitrogen content of all samples was determined by the modified Kjeldahl method with sulphuric acid, salycilic acid, sodium thiosulphate, and copper sulphate. Where nitrate nitrogen was present, 50 c. c. of concentrated sulphuric-salycilic acid (25 c. c. of concentrated acid plus 25 c. c. of distilled water) were added to the cultures slowly and with constant stirring. This acid was allowed to react for a few days, after which the usual procedure was carried out. Digestion was continued for five to six hours subsequent to the clarification of the liquid.

The amount of ammonia was determined by distillation with steam in the presence of magnesium oxid.

Nitrites (qualitative test) were tested for with Trommsdorf's reagent.

In all distillations $N/14$ acid and alkali were used.

In the preparation of agar cultures of alfalfa seedlings the seeds were treated with a 0.25 per cent solution of mercuric chlorid and rinsed in sterile distilled water. Three bacteria-free seeds were transferred to the surface of soft mannit agar (0.7 per cent agar) in each tube.

The nitrates were added in solution to all cultures. Gram-molecular quantities of potassium, sodium, calcium, and ammoniun nitrates (Merck's) were weighed into sterile distilled water. These solutions were prepared in such a manner that 5 c. c. contained 450 mgm. of nitrate. In all nitrate solutions the nitrate radical, or anion, was present in the same quantities, while the cation, or metal, was present in varying quantities, depending upon the particular salt.

Plate counts of all soil cultures were made by weighing 20 gm. (dry weight) of the soil into a 200-c. c. water blank. From this suspension all subsequent dilutions were made. Mannit agar [1] was used for the plate counts in the cultures of Azotobacter and *B. radicicola*. Duplicate plates were made for each dilution poured.

SOIL USED

Only one type of soil was employed, Miami silt loam obtained from the Experiment Station farm. No chemical analyses of the soil were made other than an estimation of its organic matter content, which was approximately 2.75 per cent. The soil was neutral in reaction and its nitrate content was approximately 1.5 mgm. of nitrogen as nitrate in 100 gm. of the dry soil.

ISOLATION OF AZOTOBACTER AND BACILLUS RADICICOLA

AZOTOBACTER.—(1) Strain A was isolated from a silt loam soil. This strain grew well on mannit agar, but produced no pigment after three weeks' growth. (2) Strain B was isolated from a sandy loam soil. This strain grew equally well on mannit agar and produced a brownish black pigment within one week's growth. Both strains assimilated practically the same amount of atmospheric nitrogen under laboratory conditions.

BACILLUS RADICICOLA.—A stock laboratory culture of *B. radicicola* was replated twice before taking the final culture. The nodule producing power of the organism was determined by inoculating bacteria-free alfalfa seedlings (in soft agar). After sufficient incubation nodules were produced in abundance.

INFLUENCE OF NITRATES ON AZOTOBACTER

INFLUENCE OF NITRATES ON THE GROWTH AND REPRODUCTION OF AZOTOBACTER IN STERILIZED SOIL

What effect do nitrates have on pure cultures of Azotobacter in sterilized soil? Do these salts cause a decrease in the numbers of the organisms? Do they cause an increase in numbers? Or do they exert no particular influence one way or the other? It is difficult to believe that the latter could be true, inasmuch as nitrates have such a profound effect on higher forms of plant life. Such readily soluble and assimilable substances as nitrates could hardly remain without affecting either an increase or a decrease in the number of organisms existing in their presence.

With the idea of determining what effect nitrates might have on Azotobacter when grown in sterilized soil, the following experiments were planned. In this work both strains of the Azotobacter (described on

[1] FRED, E. B. A LABORATORY MANUAL OF SOIL BACTERIOLOGY. p. 108. Philadelphia and London, 1916.

p. 187) were employed and conditions governing the preparation and incubation of the cultures were similar in the case of each strain. The only variation was the periods used in incubating the cultures. Counts were made after one and two weeks' incubation with strain A and after one, two, and three weeks with strain B.

TABLE I.—*Influence of potassium nitrate on the growth of Azotobacter (strain A) in sterilized soil*

Culture No.	Treatment (nitrate in 100 gm. of dry soil).	Number of organisms in 1 gm. of dry soil.				
		At beginning.	After 1 week.	Relative.	After 2 weeks.	Relative.
	Mgm.			*Per cent.*		*Per cent.*
1	0	15,600	825,000	100	315,000	100
2	0	15,600	935,000		360,000	
3	10	15,600	1,500,000	170	1,175,000	348
4	10	15,600	...		...	
5	25	15,600	4,200,000	523	12,350,000	3,418
6	25	15,600	5,000,000		10,750,000	
7	50	15,600	20,400,000	2,233	27,750,000	8,210
8	50	15,600	18,900,000		...	
9	100	15,600	11,000,000	1,295	9,000,000	2,685
10	100	15,600	11,820,000		9,150,000	
11	150	15,000	...	179	25,000	12
12	150	15,600	1,575,000		55,000	
13	200	15,600	225,000	27	0	0
14	200	15,600	250,000		0	
15	300	15,600	0	0	0	0
16	300	15,600	0		0	

TABLE II.—*Influence of sodium nitrate on the growth of Azotobacter (strain A) in sterilized soil*

Culture No.	Treatment (nitrate in 100 gm. of dry soil).	Number of organisms in 1 gm. of dry soil.				
		At beginning.	After 1 week.	Relative.	After 2 weeks.	Relative.
	Mgm.			*Per cent.*		*Per cent.*
1	0	13,800	310,000	100	425,000	100
2	0	13,800	225,000		490,000	
3	10	13,800	575,000	188	875,000	191
4	10	13,800	430,000		...	
5	25	13,800	2,850,000	1,615	2,250,000	492
6	25	13,800	5,800,000		...	
7	50	13,800	15,200,000	5,217	15,500,000	3,150
8	50	13,800	12,750,000		13,300,000	
9	100	13,800	17,750,000	6,335	9,850,000	2,800
10	100	13,800	16,200,000		15,750,000	
11	150	13,800	550,000	177	690,000	117
12	150	13,800	400,000		375,000	
13	200	13,800	0	0	0	0
14	200	13,800	0		0	
15	300	13,800	0	0	0	0
16	300	13,800	0		0	

TABLE III.—*Influence of calcium nitrate on the growth of Azotobacter (strain A) in sterilized soil*

Culture No.	Treatment (nitrate in 100 gm. of dry soil).	Number of organisms in 1 gm. of dry soil.				
		At beginning.	After 1 week.	Relative.	After 2 weeks.	Relative.
	Mgm.			*Per cent.*		*Per cent.*
1	0	10, 000	260, 000	100	310, 000	100
2	0	10, 000	330, 000		260, 000	
3	10	10, 000	5, 800, 000	1, 966	975, 000	362
4	10	10, 000	....		1, 090, 000	
5	25	10, 000	10, 700, 000	3, 440	9, 200, 000	3, 122
6	25	10, 000	9, 600, 000		8, 600, 000	
7	50	10, 000	13, 250, 000	4, 213	13, 200, 000	4, 526
8	50	10, 000	11, 600, 000		12, 600, 000	
9	100	10, 000	6, 600, 000	2, 144	8, 750, 000	2, 938
10	100	10, 000	6, 050, 000		8, 000, 000	
11	150	10, 000	3, 500, 000	1, 254	2, 000, 000	763
12	150	10, 000	3, 900, 000		2, 350, 000	
13	200	10, 000	0	0	0	0
14	200	10, 000	0		0	
15	300	10, 000	0	0	0	0
16	300	10, 000	0		0	

One hundred and fifty gm. of soil (dry weight) were weighed into 500-c. c. Erlenmeyer flasks and the nitrates added in solution, as indicated in the following tables. At the same time 1 per cent of mannit was added in solution and the moisture content was raised to approximately 18 per cent. The flasks were allowed to remain at room temperature for one day, when the contents were thoroughly mixed. The flasks and contents were then sterilized at 15 pounds' pressure for three hours. Upon cooling they were inoculated with 5 c. c. of a suspension of the organisms in sterile distilled water. The cultures were incubated at 28° C. and counts made at the intervals already indicated. Mannit agar was used in pouring the plates. Each number in the following tables represents an average of duplicate plates. Tables I, II, and III show the results of the work with strain A and Tables V, VI, and VII the results with strain B.

It will be seen at a glance that all three nitrates exerted an enormous influence on the growth of the Azotobacter. The smallest concentration did not appear to exert much influence either in increasing or decreasing the number of Azotobacter. There was a slight gain, but it was not so marked as that brought about by higher concentrations of nitrates. When 25, 50, and 100 mgm. of nitrate were present in 100 gm. of soil, very large increases were obtained in practically all instances. In one instance sodium nitrate caused the greatest relative gain, but the most consistent increase was produced by calcium nitrate. Beginning with 150 mgm. the number of Azotobacter began to decrease. This decrease was especially noticeable in the cultures containing potassium and sodium nitrates. At the end of the first week, Azotobacter organisms

were still found in the potassium-nitrate cultures where 200 mgm. were present. However, at the end of the second week the organisms were dead. The same concentration of sodium and calcium nitrates proved even more toxic. No evidences were secured, indicating that these organisms can resist concentrations in excess of 300 mgm. of nitrate per 100 gm. of soil.

The question may be raised in regard to the influence of sterilization on the nitrate present in the soil, Does the prolonged heating in the presence of soil organic matter reduce the nitrate? In order to study this point, a few cultures were prepared similar to those already described. They were subjected to sterilization under pressure of 15 pounds for two, three, and five hours. Nitrate determinations at the end of these periods failed to show any reduction. In the presence of 1 per cent of mannit the nitrate content remained unchanged during sterilization.

From these results it is evident that small amounts of nitrate up to 150 mgm. of nitrate in 100 gm. of soil greatly increased the reproduction of Azotobacter. In regard to the toxicity of higher concentrations, sodium nitrate appeared to exert the greatest influence in this direction, followed by calcium and potassium nitrates in the order named. The results of the experiment are recorded in Table IV.

TABLE IV.—*Influence of ammonium nitrate on the growth of Azotobacter (strain A) in sterilized soil*

Culture No.	Treatment (nitrate in 100 gm. of dry soil).	Number of organisms in 1 gm. of dry soil.				
		At beginning.	After 1 week.	Relative.	After 2 weeks.	Relative.
	Mgm.			*Per cent.*		*Per cent.*
1	0	18, 500	1, 400, 000	100	975, 000	100
2	0	18, 500	1, 050, 000		1, 100, 000	
3	25	18, 500	5, 600, 000	427	5, 000, 000	430
4	25	18, 500	4, 900, 000		3, 900, 000	
5	100	18, 500	2, 900, 000	223	3, 950, 000	388
6	100	18, 500	2, 600, 000		4, 100, 000	
7	200	18, 500	1, 100, 000	84	875, 000	86
8	200	18, 500	950, 000		915, 000	

That the nitrate radical and not the combined metal was the causal agent in the increase in the number of Azotobacter was indicated from the results of the next test. Here ammonium nitrate was used.

It will be seen from the data of this experiment that ammonium nitrate caused an increase in the number of Azotobacter when present in small amounts. However, the increase in the presence of ammonium nitrate was less marked than when equal quantities of the other nitrates were used. Since the experiments with ammonium nitrate were not made at the same time as the preceding experiments (discussed on pp. 189–190), it is possible that conditions varied sufficiently to account for the less pronounced results. When 200 mgm. of nitrate were present in 100 gm. of

soil the number of Azotobacter showed a decrease. Apparently ammonium nitrate is more toxic than potassium, sodium, and calcium nitrate. However, the main point at issue seems fairly well established—namely, that the increase in the number of Azotobacter is caused by the nitrate radical and not by the combined metal.

TABLE V.—*Influence of potassium nitrate on the growth of Azotobacter (strain B) in sterilized soil*

Culture No.	Treatment (nitrate in 100 gm. of dry soil).	Number of organisms in 1 gm. of dry soil.						
		At beginning.	After 1 week.	Relative.	After 2 weeks.	Relative.	After 3 weeks.	Relative.
	Mgm.			*Per cent.*		*Per cent.*		*Per ct.*
1....	0	12, 600	235, 000	100	112, 500	100	116, 000	100
2....	0	12, 600	....		110, 500		117, 000	
3....	10	12, 600	3, 750, 000	1, 510	2, 100, 000	1, 950	875, 000	916
4....	10	12, 600	3, 300, 000		2, 250, 000		1, 260, 000	
5....	25	12, 600	5, 750, 000	2, 436	1, 575, 000	1, 581	1, 700, 000	1, 300
6....	25	12, 600	5, 700, 000		1, 950, 000		1, 325, 000	
7....	50	12, 600	3, 100, 000	1, 340	3, 250, 000	3, 655	3, 525, 000	2, 783
8....	50	12, 600	3, 200, 000		4, 900, 000		2, 960, 000	
9....	100	12, 600	3, 200, 000	1, 320	4, 000, 000	3, 363	2, 500, 000	2, 317
10....	100	12, 600	3, 000, 000		3, 500, 000		2, 900, 000	
11....	150	12, 600	2, 100, 000	851	2, 000, 000	1, 838	1, 500, 000	1, 502
12....	150	12, 600	1, 900, 000		2, 100, 000		2, 000, 000	
13....	200	12, 600	875, 000	373	800, 000	695	650, 000	580
14....	200	12, 600	880, 000		750, 000		700, 000	
15....	300	12, 600	0	0	0	0	0	0
16....	300	12, 600	0		0		0	

TABLE VI.—*Influence of sodium nitrate on the growth of Azotobacter (strain B) in sterilized soil*

Culture No.	Treatment (nitrate in 100 gm. of dry soil).	Number of organisms in 1 gm. of dry soil.						
		At beginning.	After 1 week.	Relative.	After 2 weeks.	Relative.	After 3 weeks.	Relative.
	Mgm.			*Per cent.*		*Per cent.*		*Per ct.*
1....	0	15, 600	158, 000	100	110, 500	100	112, 500	100
2....	0	15, 600	149, 000		126, 000		115, 000	
3....	10	15, 600	1, 250, 000	727	1, 750, 000	1, 310	5, 000, 000	5, 097
4....	10	15, 600	990, 000		1, 350, 000		6, 600, 000	
5....	25	15, 600	1, 765, 000	1, 165	6, 600. 000	5, 029	9, 150, 000	7, 161
6....	25	15, 600	1, 825, 000		5, 300, 000		7, 150, 000	
7....	50	15, 600	1, 875, 000	1, 338	2, 025, 000	2, 141	15, 950, 000	13, 423
8....	50	15, 600	2, 250, 000		3, 040, 000		14, 600, 000	
9....	100	15, 600	2, 200, 000	1, 350	2, 775, 000	2, 525	5, 800, 000	4, 860
10....	100	15, 600	1, 950, 000		3, 200, 000		5, 250, 000	
11....	150	15, 600	165, 000	108	530, 000	556	3, 100, 000	2, 573
12....	150	15, 600	170, 000		785, 000		2, 750, 000	
13....	200	15, 600	0	0	0	0	0	0
14....	200	15, 600	0		0		0	
15....	300	15, 600	0	0	0	0	0	0
16....	300	15, 600	0		0		0	

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TABLE VII.—*Influence of calcium nitrate on the growth of Azotobacter (strain B) in sterilized soil*

Culture No.	Treatment (nitrate in 100 gm. of dry soil).	Number of organisms in 1 gm. of dry soil.						
		At beginning.	After 1 week.	Relative.	After 2 weeks.	Relative.	After 3 weeks.	Relative.
	Mgm.			*Per cent.*		*Per cent.*		*Per ct.*
1....	0	22, 000	905, 000	100	1, 475, 000	100	1, 130, 000	100
2....	0	22, 000	860, 000		1, 460, 000		1, 157, 500	
3....	10	22, 000	23, 200, 000	2, 423	28, 000, 000	2, 181	34, 050, 000	3, 002
4....	10	22, 000	19, 600, 000		36, 000, 000		34, 600, 000	
5....	25	22, 000	17, 200, 000	2, 084	52, 000, 000	3, 255	29, 750, 000	2, 273
6....	25	22, 000	19, 600, 000		43, 500, 000		22, 250, 000	
7....	50	22, 000	11, 800, 000	1, 461	22, 500, 000	1, 448	30, 400, 000	2, 633
8....	50	22, 000	14, 000, 000		20, 000, 000		29, 850, 000	
9....	100	22, 000	7, 500, 000	1, 053	12, 000, 000	818	21, 750, 000	1, 780
10....	100	22, 000	11, 000, 000		....		18, 950, 000	
11....	150	22, 000	2, 550, 000	342	5, 300, 000	402	4, 800, 000	420
12....	150	22, 000	3, 500, 000		6, 500, 000		....	
13....	200	22, 000	107, 500	11	2, 750, 000	203	130, 000	11
14....	200	22, 000	87, 500		3, 225, 000		120, 000	
15....	300	22, 000	0	0	0	0	0	0
16....	300	22, 000	0		0		0	

A glance at the figures of Tables V, VI, and VII shows that the smallest concentration of nitrate used produced a much more marked relative increase in numbers with strain B than it did with strain A. On the other hand, the greater resistance of this strain to the higher nitrate concentrations is clearly evident. In the potassium- and calcium-nitrate cultures the organisms were present in an active state where the nitrate was added in amounts equivalent to 200 mgm. of nitrate in 100 gm. of soil. However, this same concentration of sodium nitrate prevented the development of the Azotobacter. The first five concentrations of all three nitrates caused a very large increase in the number of Azotobacter when compared with control cultures where no nitrate was added. In one instance an enormous increase was noted after three weeks' incubation in the presence of 50 mgm. of nitrate as sodium nitrate. This increase far excelled that noted with other concentrations of the same salt. The writer can offer no conjecture as to this occurrence.

Similar results were obtained by the writer in 1914 (*23*) with a strain of Azotobacter isolated from a silt loam soil at the Pennsylvania Experiment Station. It was found that soil and liquid cultures containing small amounts of potassium, sodium, and calcium nitrates caused an increase in the number of Azotobacter in pure culture compared with control cultures containing no nitrate. An increasing concentration of the nitrates continued favorable to the growth of the organism up to a certain limit, but higher concentrations retarded its growth. Finally a nitrate concentration was attained at which Azotobacter growth altogether ceased.

The results of the study of nitrates and their influence on Azotobacter in sterilized soil show very clearly that small amounts of nitrate cause a great increase in the number of Azotobacter cells. Higher concentrations are not so favorable to the growth of the organisms, and the highest concentrations studied prevented the development of the Azotobacter in sterilized soil.

From a study of the results of these experiments, it seems that the increase in number of Azotobacter in the presence of small amounts of nitrate is a direct result of nuclear stimulation. Later studies to be cited (*pp. 205–208*) show that nitrates exerted considerable influence on the internal structure of the Azotobacter cell. It appears reasonable to expect that the nitrate affected the nuclear structure in such a manner that an increase in cell multiplication resulted. It seems probable that the action of nitrate as a simple nutrient would be shown by a slower increase in cell multiplication.

INFLUENCE OF NITRATES ON THE FIXATION OF NITROGEN BY AZOTOBACTER

It has been shown in the preceding paragraphs that the presence of small quantities of nitrate in sterilized soil bring about a large increase in the number of Azotobacter. This increase was noted in the case of both strains of Azotobacter. It would be of interest to know whether the increase in bacterial numbers was accompanied by a corresponding increase in the amount of nitrogen assimilated.

The results secured by a few investigators indicate that in the presence of combined nitrogen as nitrates the nonsymbiotic nitrogen-fixing organisms will not fix atmospheric nitrogen. Stoklasa (*44, p. 492–503*) studied the influence of Azotobacter on sodium nitrate in aerobic and anaerobic liquid cultures. He found only a small gain in organic nitrogen and from these results he concluded that in the presence of nitrates Azotobacter could not assimilate atmospheric nitrogen. It has been shown by Hanzawa (*20*) that in a liquid culture containing 12 mgm. of nitrate (from potassium nitrate) in 100 c. c. of medium, a mixed culture of Azotobacter fixed 5.25 mgm. of nitrogen. Under the same conditions with 60 mgm. of nitrate present in 100 c. c. of medium he found but 5.35 mgm. of nitrogen fixed. He concluded that nitrates remained, as far as small quantities were concerned, almost without influence on the amount of atmospheric nitrogen fixed by Azotobacter.

Some studies have been carried on with respect to the influence of nitrates on the nonsymbiotic anaerobic nitrogen-assimilating organism, *Clostridium* spp. Bredemann (*9*) showed that ammonium nitrate in solution caused a decrease in the amount of nitrogen fixed by species of Clostridium. Pringsheim (*40*) grew cultures of *C. americanum* in solutions containing potassium nitrate. He found that in the presence of available energy the organism fixed some nitrogen when nitrate was

present but to a less extent than did control cultures containing no nitrate.

From these results it appears that nitrates do not stimulate the nitrogen-assimilation of the nonsymbiotic nitrogen-fixing bacteria.

Inasmuch as nitrates in small amounts caused such an increase in the number of Azotobacter in sterilized soil, it was thought advisable to determine just what influence these salts exert on nitrogen fixation by Azotobacter. Accordingly, experiments were carried out with Azotobacter on agar films, in soil cultures and in solution.

AGAR-FILM CULTURES.—In this work both strains of the Azotobacter were used. One hundred c. c. of mannit agar were placed in liter Erlenmeyer flasks and nitrates of potassium, sodium and calcium added in varying quantities. The flasks and contents were sterilized at 10 pounds' pressure for 25 minutes, cooled, and inoculated with 10 c. c. of a suspension of the organism in sterile distilled water. The flasks were incubated at 28° C. for three weeks. The weight of both inoculated and uninoculated flasks was maintained throughout the experiment by the addition of sterile distilled water. At the end of the incubation period total nitrogen analyses were made. Because of the high nitrate content dilute sulphuric-salycilic acid was added slowly and carefully to prevent loss of nitrogen by the evolution of gaseous oxids of nitrogen. The acid was allowed to react for a few days before continuing the total nitrogen determination. The results of the experiments are presented in Tables VIII and IX.

TABLE VIII.—*Influence of nitrates on the fixation of nitrogen by Azotobacter (strain A) on agar films*

Culture No.	Treatment (nitrate in 100 c. c. of medium).	Nitrogen contained in 100 c. c. of medium. Inoculated. Found.	Inoculated. Average.	Uninoculated. Found.	Uninoculated. Average.	Nitrogen fixed.
		Mgm.	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*
1	0	13. 00		4. 0		
2	0	12. 70	12. 80	4. 0	4. 05	8. 75
3	0	12. 60		4. 1		
4	50 mgm. of NO_3 potassium nitrate	18. 50	18. 45	7. 00	7. 10	11. 35
5	....do	18. 40		7. 20		
6	100 mgm. of NO_3 potassium nitrate	27. 60	27. 70	16. 80	16. 25	11. 45
7	....do	27. 75		15. 70		
8	50 mgm. of NO_3 sodium nitrate	18. 65	18. 50	7. 50	7. 4	11. 10
9	....do	18. 30		7. 30		
10	100 mgm. of NO_3 sodium nitrate	27. 00	27. 35	15. 00	15. 1	12. 25
11	....do	27. 65		15. 20		
12	50 mgm. of NO_3 calcium nitrate	13. 75	13. 75	8. 00	8. 25	5. 50
13	....do	13. 70		8. 50		
14	100 mgm. of NO_3 calcium nitrate	18. 80	18. 95	14. 50	14. 40	4. 55
15	....do	19. 15		14. 30		

TABLE IX.—*Influence of nitrates on the fixation of nitrogen by Azotobacter (strain B) on agar films*

Culture No.	Treatment (nitrate in 100 c. c. of medium).	Nitrogen contained in 100 c. c. of medium.				Nitrogen fixed.
		Inoculated.		Uninoculated.		
		Found.	Average.	Found.	Average.	
		Mgm.	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*
1	0	15. 50	15. 60	6. 50	6. 40	9. 20
2	0	15. 70		6. 30		
3	0	15. 60		6. 40		
4	75 mgm. of NO_3 as potassium nitrate	25. 20	25. 30	13. 00	13. 85	11. 45
5	....do	25. 40		14. 70		
6	150 mgm. of NO_3 as potassium nitrate	36. 40	36. 65	24. 00	23. 60	13. 05
7	....do	36. 90		23. 20		
8	75 mgm. of NO_3 as sodium nitrate	25. 60	25. 65	12. 80	13. 00	12. 65
9	....do	25. 70		13. 20		
10	150 mgm. of NO_3 as sodium nitrate	37. 60	37. 40	26. 20	25. 80	11. 60
11	....do	37. 20		25. 40		
12	75 mgm. of NO_3 as calcium nitrate	20. 10	19. 85	12. 00	12. 35	7. 50
13	....do	19. 60		12. 70		
14	150 mgm. of NO_3 as calcium nitrate	32. 80	33. 05	24. 50	24. 85	8. 20
15	....do	33. 30		25. 20		

A glance at the results (Tables VIII and IX) shows that an increase in nitrogen fixation occurred where potassium and sodium nitrates were present, whereas a marked decrease in the total nitrogen content was observed where calcium nitrate was used. Whether the calcium itself is detrimental to an increase in organic nitrogen or whether it is the combination of calcium with nitrate can not be stated. It is significant, however, that this decrease in fixation of nitrogen was noted throughout all the experiments where calcium nitrate was employed. It is very evident that calcium nitrate exerts some detrimental effect on the nitrogen assimilating properties of the organism.

There seems to be but a slight difference in the nitrogen-fixing ability of the two strains studied. In the absence of nitrates the amount fixed varies but little. Also in the presence of potassium and sodium nitrates the relative increase in amount of nitrogen fixed remains about the same. Calcium nitrate offers an exception where it is employed. The detrimental effect seems to be more marked in the case of strain A than with strain B. Strain A under normal conditions fixed slightly less nitrogen than strain B, so it may be possible that this strain is weaker.

The formation of pigment by the Azotobacter in the presence of the nitrates is of interest. Strain A normally produced no pigment by the end of three weeks' incubation. But when grown on the agar films in the presence of nitrate a most marked pigment production appeared. This pigment was especially noticeable in the presence of the calcium

salt. Since strain B normally produces a good pigment, the influence of nitrate on this strain was not very marked. The relation of nitrates to pigment formation will be taken up later (pp. 203–205).

From the results of the experiments with agar films containing various amounts of nitrate, it seems apparent that potassium and sodium nitrates in amounts of 50 and 100 mgm. of nitrate in 100 c. c. of medium cause a small increase in the amount of nitrogen fixed. However, this increase in fixation is not at all parallel with the increase in number of Azotobacter caused by nitrates in sterilized soil.

It may be concluded that an increase in the number of Azotobacter in sterilized soil as a result of nitrate stimulation does not mean a corresponding increase in nitrogen fixation on agar films.

Soil cultures.—The conditions obtaining in these experiments were strictly comparable with those heretofore cited dealing with the influence of nitrates on Azotobacter in sterilized soil (pp. 187–193).

The fixation of nitrogen was studied in pure culture in sterilized soil and in unsterilized soil. One hundred and fifty gm. of soil (dry weight) were weighed into 1-liter Erlenmeyer flasks, nitrates were added in varying amounts from 10 to 200 mgm., and 1 per cent of mannit was also added. Triplicate flasks were prepared for each amount of nitrate studied. The moisture content was raised to approximately 18 per cent and the flasks allowed to remain at room temperature for one day. The contents were then thoroughly mixed and a fine crumb structure produced. The flasks for the experiments with pure cultures in sterilized soil were immediately sterilized at 15 pounds' pressure for three hours. After cooling, two of each set were inoculated with 5 c. c. of a suspension of Azotobacter (strain A) in sterile distilled water. The remaining flask of each set was not inoculated, but was incubated at 28° C. with the inoculated flasks. The moisture lost by evaporation was replaced from time to time by the addition of sterile distilled water. At the end of the incubation period the soil was removed and spread out in thin layers and allowed to dry. It was then thoroughly ground in a porcelain-ball mill for one hour. At the end of this time all of the soil passed through a 100-mesh sieve.

Soil cultures used in the study of the effect of nitrates on nitrogen fixation in unsterilized soil were prepared in a similar manner, except that the flasks were not sterilized. Previous to incubation a small inoculum of Azotobacter (strain A) was added to insure the presence of the nitrogen-fixing organism in the soil cultures. The proper moisture content was maintained in the same manner as in the case of the pure cultures in sterilized soil and the incubation period was the same for both. The results are given in Tables X, XI, XII, and XIII.

TABLE X.—*Influence of sodium nitrate on the fixation of nitrogen by Azotobacter in sterilized soil*

Culture No.	Treatment (nitrate in 100 gm. of dry soil).	Total nitrogen in 100 gm. of dry soil.				Nitrogen fixed in 100 gm. of dry soil.
		Inoculated.		Uninoculated.		
		Found.	Average.	Found.	Average.	
	Mgm.	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*
1	0	135.0		132.0		
1	0	134.0	133.7	131.5	131.5	2.7
1	0	132.0		131.0		
2	0	133.0		....	....	
2	0	137.0	135.0	....	....	3.5
2	0	....		....	....	
3	10	137.0		134.0		
3	10	136.0	136.6	133.5	133.7	2.9
3	10	137.0		134.0		
4	10	136.5		....	....	
4	10	137.5	137.0	....	....	3.3
4	10	137.0		....	....	
5	50	149.0		140.0		
5	50	149.0	149.0	137.0	138.5	10.5
5	50	149.0		138.5		
6	50	148.5		....	....	
6	50	149.5	149.2	....	....	10.7
6	50	149.5		....	....	
7	150	163.0		152.0		
7	150	162.0	162.3	150.0	151.5	10.8
7	150	162.0		152.5		
8	150	162.5		....	....	
8	150	163.0	162.5	....	....	11.0
8	150	162.0		....	....	

TABLE XI.—*Influence of sodium nitrate on the fixation of nitrogen by Azotobacter in unsterilized soil*

Culture No.	Treatment (nitrate in 100 gm. of dry soil).	Total nitrogen in 100 gm. of dry soil.				Nitrogen fixed in 100 gm. of dry soil.
		Inoculated.		Uninoculated.		
		Found.	Average.	Found.	Average.	
	Mgm.	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*
1	0	132.0		130.0		
1	0	135.0	134.0	133.5	131.8	2.2
1	0	135.0		132.0		
2	0	132.0		....	....	
2	0	134.0	133.3	....	....	1.5
2	0	134.0		....	....	
3	10	137.5		134.0		
3	10	138.8	137.8	133.0	133.3	4.5
3	10	138.8		133.0		
4	10	137.5		....	....	
4	10	137.5	137.7	....	....	4.4
4	10	138.0		....	....	
5	50	150.0		140.0		
5	50	151.0	150.3	140.5	140.8	9.5
5	50	150.0		142.0		
6	50	149.0		....	....	
6	50	149.5	149.7	....	....	8.9
6	50	150.5		....	....	
7	150	169.0		148.0		
7	150	167.0	168.0	154.0	151.8	16.2
7	150	168.0		153.5		
8	150	167.5		....	....	
8	150	168.0	168.0	....	....	16.2
8	150	168.5		....	....	

TABLE XII.—*Influence of calcium nitrate on the fixation of nitrogen by Azotobacter in sterilized soil*

Culture No.	Treatment (nitrate in 100 gm. of dry soil).	Total nitrogen in 100 gm. of dry soil.				Nitrogen fixed in 100 gm. of dry soil.
		Inoculated.		Uninoculated.		
		Found.	Average.	Found.	Average.	
	Mgm.	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*
1	0	133.0		131.0		
1	0	133.6	133.3	131.0	131.3	2.0
1	0	133.3		132.0		
2	0	133.0		....	....	
2	0	134.2	133.5	....	....	2.2
2	0	133.4		....	....	
3	10	137.0		135.0		
3	10	137.0	136.8	134.0	134.7	2.1
3	10	136.5		135.0		
4	10	136.5		....	....	
4	10	137.0	137.0	....	....	2.3
4	10	137.5		....	....	
5	50	148.0		140.5		
5	50	148.5	148.5	141.0	140.7	7.8
5	50	149.0		140.5		
6	50	148.5		....	....	
6	50	149.0	148.5	....	....	7.8
6	50	148.0		....	....	
7	200	173.0		163.0		
7	200	173.0	173.7	164.0	163.8	9.9
7	200	174.0		164.5		
8	200	173.5		....	....	
8	200	173.0	173.5	....	....	9.7
8	200	174.0		....	....	

TABLE XIII.—*Influence of calcium nitrate on the fixation of nitrogen by Azotobacter in unsterilized soil*

Culture No.	Treatment (nitrate in 100 gm. of dry soil).	Total nitrogen in 100 gm. of dry soil.				Nitrogen fixed in 100 gm. of dry soil.
		Inoculated.		Uninoculated.		
		Found.	Average.	Found.	Average.	
	Mgm.	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*
1	0	134.5		134.0		
1	0	136.0	135.7	133.5	133.2	2.5
1	0	136.5		132.0		
2	0	135.0		....	....	
2	0	135.5	135.3	....	....	2.1
2	0	135.5		....	....	
3	10	138.5		133.5		
3	10	138.0	138.5	133.0	133.2	5.3
3	10	139.0		133.0		
4	10	138.0		....	....	
4	10	137.5	138.0	....	....	4.8
4	10	138.5		....	....	
5	50	151.5		140.5		
5	50	152.0	151.5	141.0	141.0	10.5
5	50	151.0		141.5		
6	50	150.0		....	....	
6	50	151.5	150.8	....	....	9.8
6	50	151.0		....	....	
7	200	177.0		164.0		
7	200	178.0	177.0	165.0	164.3	12.7
7	200	176.0		164.0		
8	200	176.5		....	....	
8	200	177.0	177.2	....	....	12.9
8	200	178.0		....	....	

It will be seen at a glance that a greater relative increase in nitrogen fixation in the presence of nitrates occurred in the soil cultures than on the agar films. But in the latter instance the amount of nitrogen assimilated in the absence of mistakes is far in excess of that assimilated in the soil cultures under similar conditions. The amount of nitrogen fixed in the soil cultures is surprisingly low, but as relative increases or decreases are desired this does not materially influence the results.

The influence of sodium nitrate on the fixation of nitrogen by pure cultures of Azotobacter in sterilized and unsterilized soil is brought out very clearly in the figures of Tables X and XI. In both cases, where no nitrate was added, an equal fixation of nitrogen occurred. Where 10 mgm. of nitrate were added to 100 gm. of soil, slightly more nitrogen was assimilated in the unsterilized soil than in sterilized. The reverse seemed to be true when 50 mgm. of nitrate were added. But in the presence of 150 mgm. of nitrate, the fixation by the pure culture in sterilized soil did not increase materially in comparison with that which occurred in the 50 mgm. of nitrate concentration. Evidently the maximum fixation under these conditions had been reached. The gain in the unsterilized soil at the highest concentration of nitrate studied almost doubled the amount fixed in the pure culture. It appears evident that the presence of sodium nitrate causes a greater fixation of nitrogen in unsterilized soil than it does under similar conditions in sterilized soil inoculated with Azotobacter.

In the case of calcium nitrate, somewhat comparable results were obtained. The fixation where no nitrate was added was equivalent to that obtained in the controls for the sodium nitrate. Where nitrate was added in amounts equal to 10 mgm. of nitrate in 100 gm. of soil, an increased fixation was obtained in the unsterilized soil, but practically no increase occured in the pure culture in sterilized soil. Fifty mgm. of nitrate in 100 gm. of soil produced an increase in fixation. In the highest concentration of calcium nitrate the difference in nitrogen fixed between the pure culture in sterilized soil and unsterilized soil was not so great as in the case where sodium nitrate was used.

In the sterilized soil where the two nitrates were present in equal amounts it can be seen that more fixation took place in the presence of sodium nitrate. The difference is not marked, but it exists nevertheless. It will be remembered that calcium nitrate had a detrimental effect on nitrogen fixation by Azotobacter on agar films. However, in soil cultures this same nitrate stimulated Azotobacter to an increased assimilation of nitrogen. This difference is not suprising as it has been shown repeatedly that bacterial activities in soil and in artificial cultures are not always comparable.

From the results of the experiments performed with reference to the influence of nitrates in soil on the fixation of nitrogen therein, it appears

evident that in pure cultures both sodium and calcium nitrates in the amounts studied produced an increase in the amount of nitrogen fixed. The sodium salt stimulated this process to a slightly greater extent than did the calcium salt. In unsterilized soil nitrates exerted the same action but to a more marked extent. The amount of nitrogen fixed under these conditions was generally in excess of that fixed under similar conditions in sterilized soil inoculated with a pure culture of Azotobacter.

Such large relative increases in total nitrogen in the soil in the presence of nitrates would not normally take place under field conditions for here no accumulations of nitrate occur in quantities sufficiently large enough to influence this process.

Summing up all the experiments performed in relation to the influence of nitrates on the fixation of atmospheric nitrogen by Azotobacter, it appears that the increase in total nitrogen in the presence of these salts is by no means comparable to the increase in the number of organisms in sterilized soil under the same conditions. An increase in the number of Azotobacter does not mean a parallel increase in the amount of nitrogen fixed.

INFLUENCE OF AZOTOBACTER ON NITRATES IN SOLUTION

Attention has been thus far directed toward the influence exerted by nitrates on the growth and nitrogen-assimilating power of Azotobacter. The following points are now to be considered: Do the nitrogen-fixing bacteria reduce nitrates to nitrites and ammonia? Is there an increase or decrease in the amount of organic nitrogen as a result of the presence of nitrate in the medium?

Beijerinck and Van Delden (5) found that *Azotobacter chroococcum* reduced nitrate directly to ammonia. Stoklasa (*44, p. 492-503*) studied the changes in a nutrient solution containing 0.2 per cent of sodium nitrate inoculated with Azotobacter. He found under anærobic conditions that the nitrate was largely reduced to nitrite and ammonia and that a very small amount of organic nitrogen was formed. Under ærobic conditions there was more nitrite formed than under anærobic conditions and very little ammonia or oganic nitrogen. He concluded, therefore, that Azotobacter did not fix atmospheric nitrogen in the presence of nitrates.

The following experiments were performed in an endeavor to answer the questions raised in the initial paragraph of this section. To Erlenmeyer flasks of 500-c. c. capacity, containing 100-c. c. portions of mannit solution, sodium and ammonium nitrates were added in amounts equivalent to 150 mgm. of nitrate in 100 c. c. of the solution. Nine flasks were prepared for each nitrate and the same number for the controls containing no nitrate. The flasks and contents were sterilized at 10 pounds pressure for 30 minutes. After cooling, six of each set were inoculated, three

with strain A and three with strain B, and the remaining three were left uninoculated to serve as controls. The flasks were incubated at 28° C. for 21 days. The total weight was maintained throughout the experiment by the addition of sterile distilled water from time to time. At the end of three weeks the contents of each set of triplicate flasks were poured together and 50-c. c. samples drawn for analysis. Nitrate ammonia and total nitrogen were determined as given under "Methods." The results are shown in Tables XIV, XV, and XVI.

TABLE XIV.—*Influence of Azotobacter on nitrates in solution, giving the quantity of nitrate lost*

Culture No.	Treatment (nitrate in 100 c. c. of medium).	Nitrate in 100 c. c. of medium.									
		Strain A.					Strain B.				
		Inoculated.		Uninoculated.		Nitrate lost.	Inoculated.		Uninoculated.		Nitrate lost.
		Found.	Average.	Found.	Average.		Found.	Average.	Found.	Average.	
		Mgm.	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*
1–9	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1–9	0	.00		.00			.00		.00		
10–18	150 gm. of NO_3 as sodium nitrate	80.9	80.75	150.4	150.8	[a]—70.05	105.6	105.4	150.4	150.8	[b]—45.40
10–18	.....do	80.6		151.3			105.2		151.3		
19–27	150 mgm. of NO_3 as ammonium nitrate	100.3	101.2	149.6	149.8	[a]—48.60	131.1	130.9	149.6	149.8	[c]—18.90
19–27	.....do	102.1		150.0			130.7		150.0		

[a] Strong NO_2 reaction. [b] Medium NO_2 reaction. [c] Slight NO_2 reaction.

TABLE XV.—*Influence of Azotobacter on nitrates in solution, giving the quantity of ammonia produced*

Culture No.	Treatment (nitrate in 100 c. c. of medium).	Nitrogen as ammonia in 100 c. c. of medium.									
		Strain A.					Strain B.				
		Inoculated.		Uninoculated.		Ammonia produced.	Inoculated.		Uninoculated.		Ammonia produced.
		Found.	Average.	Found.	Average.		Found.	Average.	Found.	Average.	
		Mgm.	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*
1–9	0	0.20	0.15	0.00	0.00	0.15	0.00	0.10	0.00	0.00	0.10
1–9	0	.10		.00			.20		.00		
10–18	150 mgm. of NO_3 as sodium nitrate	2.00	1.90	—.10	.05	1.85	2.20	2.30	—.10	.05	2.25
10–18	.....do	1.80		.20			2.40		.20		
19–27	150 mgm. of NO_3 as ammonium nitrate	13.90	13.97	13.90	13.90	.07	13.80	13.80	13.90	13.90	.10
19–27	.....do	13.95		13.90			13.80		13.90		

TABLE XVI.—*Influence of Azotobacter on nitrates in solution, giving the quantity of nitrogen fixed*

Culture No.	Treatment (nitrate in 100 c. c. of medium).	Total nitrogen in 100 c. c. of medium.									
		Strain A.					Strain B.				
		Inoculated.		Uninoculated.		Nitrogen fixed.	Inoculated.		Uninoculated.		Nitrogen fixed.
		Found.	Average.	Found.	Average.		Found.	Average.	Found.	Average.	
		Mgm.	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*
1–9	0	5.00	5.00	2.60	2.65	2.35	5.00	5.05	2.60	2.65	2.40
1–9	0	5.00		2.70			5.10		2.70		
10–18	150 mgm. of NO_3 as sodium nitrate	22.50	22.55	14.00	14.10	8.45	28.00	27.90	14.00	14.10	13.80
10–18	do	22.60		14.20			27.80		14.20		
19–27	150 mgm. of NO_3 as ammonium nitrate	47.00	46.95	43.20	43.05	3.90	48.10	48.15	43.20	43.05	5.10
19–27	do	46.90		42.90			48.20		42.90		

Table XIV showing the effect on the total nitrate content will be discussed first. Strain A differed widely from strain B in its ability to reduce nitrates. It will be noted that strain A reduces nitrate more readily than strain B in the presence of both sodium and ammonium nitrate. In order to determine the nature of the reduction of the nitrates, qualitative and quantitative tests were made. The reduction of nitrates by Azotobacter takes place with the formation of nitrites as shown in Table XIV. Strain A effected a strong reduction of nitrate to nitrite with both sodium and ammonium nitrate. Strain B also reduced nitrate to nitrite, but to a lesser degree than did strain A.

An inspection of the data in Table XV indicates that the reduction of nitrates ceased with the formation of nitrite, since no appreciable amounts of ammonia were produced by either strain of Azotobacter.

In regard to the fixation of atmospheric nitrogen by these strains of Azotobacter, it was found that nitrogen was assimilated both in the presence and absence of nitrate. In the presence of nitrate there was a large increase in the total organic nitrogen. Sodium nitrate stimulated both strains, although strain B fixed the larger amount. Similar results were obtained when the fixation of nitrogen on agar films was studied. In the presence of ammonium nitrate the amount of nitrogen fixed was considerably decreased, but the amount fixed was in excess of the control cultures containing no nitrate. It seems evident that sodium and ammonium nitrate in the amounts studied did not prevent the fixation of atmospheric nitrogen. In fact, the presence of these salts seemed to stimulate the process.

Under aerobic conditions both strains of Azotobacter studied caused a reduction in the total amount of nitrate present in the solution. This reduction may be accounted for in two ways: (1) The reduction of nitrate to nitrite and (2) the assimilation of nitrate by the organisms. Practically no ammonia was formed under the conditions of these experiments. These results agree with those of Stoklasa. However, in con-

trast to the work of Stoklasa, both strains of Azotobacter assimilated more atmospheric nitrogen in the presence of nitrates in solution than in the absence of these salts.

INFLUENCE OF NITRATES ON THE PRODUCTION OF PIGMENT BY AZOTOBACTER

It has already been noted in the experiments dealing with the effect of nitrates on the fixation of atmospheric nitrogen on agar films that nitrates favor pigment production. This was true in the case of both strains of the Azotobacter.

Moreover, it has been observed by other investigators that Azotobacter when grown in the presence of nitrate will produce a darker pigment than when grown in its absence. Beijerinck (*4, p. 575*) states that Azotobacter in pure culture will form a dark-brown pigment in the presence of glucose and a small amount of nitrate. Sackett (*43*) found that nitrate caused an increase in pigment production by Azotobacter. In media without the nitrate the pigment formation was materially decreased and in some cases practically eliminated. He also noted that the amount of nitrate present has a direct influence on the intensity of the pigment formation. He found that when sodium nitrate was added to a suitable medium to give a content of 0.0, 0.01, 0.03, 0.05, 0.08, 0.1, 0.3, and 0.5 per cent, with glucose used as the source of energy, the organisms produced pigment. Streak inoculations were made, and after 14 days' incubation he found that the maximum of color was obtained at 0.05 to 0.08 per cent and that greater concentrations did not increase the intensity of the brown-black pigment. From his results it is evident that sodium nitrate caused an increase in pigment formation by azotobacter.

In order to determine the possible effect of potassium, sodium, and calcium nitrate on pigment formation with strains A and B, the following experiment was performed.

Under normal conditions on mannit agar free from nitrate strain A produced little or no pigment even after three weeks' growth. At the end of this time dirty-yellow streaks occurred throughout the growth, but no brown pigment was produced. However, with strain B at the end of two or three weeks a decided brown to brown-black pigment was produced in the absence of nitrate.

Agar slope cultures containing increasing amounts of potassium, sodium, and calcium nitrate, as indicated in Table XVII, were prepared. These were inoculated with both strains of Azotobacter and incubated at 28° C. for 10 days. Daily observations were made for first evidences of pigment formation. In some of the cultures of strain A growing on media containing calcium nitrate this pigmentation was observed as early as 48 hours subsequent to inoculation. The following day pigmentation developed in strain B. The cultures on the potassium and sodium-nitrate media began to show evidence of pigmentation in four to six days. The final results, obtained after 10 days' incubation, are found in Table XVII.

TABLE XVII.—*Influence of nitrates on the formation of pigment in Azotobacter*

Culture No.	Treatment (Nitrate in 100 c. c. of medium).	Relative increase in pigment formation.					
		Strain A.			Strain B.		
		Potassium nitrate.	Sodium nitrate.	Calcium nitrate.	Potassium nitrate.	Sodium nitrate.	Calcium nitrate.
	Mgm.						
1	0	None	None	None	Medium brown	Medium brown	Medium brown.
2	10	Very light brown	Very light brown	Very light brown	do	do	Dark brown.
3	25	Light brown	do	Light brown	do	do	Medium brown.
4	50	do	Light brown	Medium brown	do	do	Do.
5	100	Medium brown	do	do	do	do	Do.
6	150	do	Medium brown	Dark brown	do	do	Dark brown.
7	200	do	do	do	do	Dark brown	Brown-black.
8	300	do	do	Brown-black	Dark brown	do	Do.

A general idea may be gained from Table XVII concerning the relative increase in pigment formation in the presence of the nitrates. A study of the table gives a fair idea of the relative differences in pigment production.

Very interesting results were obtained with strain A. It will be seen from Table XVII that no pigment was produced in the control culture after 10 days, while in the presence of nitrates pigmentation was noted. The intensity of the pigment varied with the increase of nitrate up to 150 mgm. Beyond 150 mgm. there was no increase. Potassium and sodium nitrate did not exert such a decided influence on pigment production as calcium nitrate. The latter salt produced an intense dark-brown to brownish-black pigment.

In the case of strain B the influence of nitrate was not so pronounced since this strain normally produced considerable pigment in the absence of nitrates. Potassium and sodium nitrate caused a slight increase in pigment formation. Here, again, the calcium salt brought about most pronounced increase. However, the relative increase in pigment formation in strain B was not so pronounced as in strain A.

Where the nitrate was present, a much more spreading growth was obtained. A heavy bacterial growth accumulated at the base of the slope except in the two cultures in which the highest concentrations were used. In the latter instances the accumulation was less than those in cultures growing on media containing no nitrate. Although the original inoculation could not be made absolutely uniform, so far as number of organisms was concerned; nevertheless it was evident that on those slopes containing 10, 25, 50, and 100 mgm. of nitrate in 100 c. c. of the medium a much more abundant growth was obtained than on those slopes free from nitrate. Here, again, it is seen, in a rough, comparative way, that the smaller amounts of nitrates caused an increase in the number of Azotobacter.

The results of this work on pigment production are quite in accord with those of Sackett. Potassium, sodium, and especially calcium, nitrates in varying amounts increase pigment formation by Azotobacter with an increase in nitrate concentration. This effect is especially marked in strain A, which under normal conditions does not produce any pigment.

INFLUENCE OF NITRATES ON THE FORMATION OF VOLUTIN BODIES IN AZOTOBACTER

The presence of volutin bodies, or metachromatic granules, in Azotobacter has been shown by Bonazzi (7). These substances, according to Meyer (*34, p. 238*), are reserve food materials other than fat droplets, glycogen, and similar substances reacting with iodin stain which occur in the cytoplasm of the cells of various bacteria. With Millon's reagent they give no reaction. He believes that these bodies are composed of nucleic-acid compounds, but are not nuclear proteids.

In connection with the foregoing investigations concerning the influence of nitrates on pigment formation by Azotobacter, it was thought that some results of cytological interest might be obtained in regard to the effect of varying amounts of nitrates on the volutin bodies.

Slope cultures of mannit agar were prepared containing the different nitrates as indicated in Table XVIII. These slopes were inoculated with both strains of Azotobacter and incubated at 28° C. for 10 days. At the end of this time each culture was stained and examined microscopically. The following method was used for demonstrating the presence of the volutin bodies. The organisms to be examined were air dried on a glass slide and then fixed in the flame of a Bunsen burner. The preparation was then flooded with a 1 to 10 aqueous solution of methylene blue (Merck's) prepared by adding 10 c. c. of a saturated aqueous solution of methylene blue to 90 c. c. of distilled water. The stain was washed off after five minutes with a 1 per cent solution of sulphuric acid and immediately rinsed in distilled water. The preparation was dried and examined with the oil-immersion objective. The volutin bodies appeared within the cytoplasm as very dark blue dots, the outline of the cell wall was a lighter blue, while the cell net work was stained a very light blue.

Guignard's stain [1] was also used to demonstrate the presence of the volutin bodies. Fresh smears on a glass slide were fixed over 10 per cent osmic acid for three minutes. The preparation was then air-dried and fixed to the slide by rapidly passing the latter a few times through a Bunsen burner. The preparation was covered with the stain which was allowed to react for five minutes. The stain was then washed off with distilled water, dried, and examined with the oil-immersion objective. The outline of the cell as well as the net work within-was stained light purple. The granules within the cytoplasm were a reddish purple. The results are given in Table XVIII.

TABLE XVIII.—*Influence of nitrates on the formation of volutin bodies in Azotobacter in 10 days*

Culture No.	Treatment (nitrate in 100 c. c. of medium).	Strain A.			Strain B.		
		Potassium nitrate.	Sodium nitrate.	Calcium nitrate.	Potassium nitrate.	Sodium nitrate.	Calcium nitrate.
	Mgm.						
1	0	Present.[a]	Doubtful	Doubtful	Present [a]	Doubtful	Present.[a]
2	10	do.[a]	Present [a]	Present [a]	do.[a]	Present [a]	Do.[a]
3	25	do.[a]	do.[a]	do.[a]	do.[a]	do.[b]	Do.[a]
4	50	do.[a]	do.[b]	Doubtful	do.[a]	do.[a]	Do.[a]
5	100	do.[a]	do.[b]	Present [a]	do.[b]	do.[b]	Do.[a]
6	150	do.[a]	do.[b]	do.[a]	do.[b]	do.[b]	Do.[b]
7	200	do.[b]	do.[b]	do.[b]	do.[b]	do.[b]	Do.[a]
8	300	do.[b]	do.[b]	do.[a]	do.[b]	do.[b]	Do.[b]

[a] Representing an approximate average of two volutin bodies per cell.
[b] Representing an approximate average of four volutin bodies per cell.

[1] Guignard'sstain. Fifty c. c. of 2 per cent fuchsin in 1 per cent acetic acid; 40 c. c. of 0.2 per cent methyl green in 1 per cent acetic acid; 1 c. c. of glacial-acetic acid. Distilled water was used in making the 1 per cent acetic-acid solution.

TABLE XXX.—*Influence of nitrates on alfalfa roots and nodule formation*

Culture No.	Treatment (nitrate in 100 c. c. of medium).	Total number of nodules in each tube of seedlings inoculated after—			
		5 days' growth.	10 days' growth.	18 days' growth.	22 days' growth.
1	None	3	3	5	4
2	10 mgm. of NO_3 as potassium nitrate	0	1	2	0
3	25 mgm. of NO_3 as potassium nitrate	0	0	0	0
4	50 mgm. of NO_3 as potassium nitrate	0	0	0	(a)
5	100 mgm. of NO_3 as potassium nitrate	0	0	0	0
6	150 mgm. of NO_3 as potassium nitrate	0	0	(b)	0
7	10 mgm. of NO_3 as sodium nitrate	0	1	3	2
8	25 mgm. of NO_3 as sodium nitrate	0	0	0	0
9	50 mgm. of NO_3 as sodium nitrate	0	0	0	0
10	100 mgm. of NO_3 as sodium nitrate	(b)	0	0	0
11	150 mgm. of NO_3 as sodium nitrate	(b)	0	0	0
12	10 mgm. of NO_3 as calcium nitrate	1	3	1	0
13	25 mgm. of NO_3 as calcium nitrate	0	0	0	0
14	50 mgm. of NO_3 as calcium nitrate	0	0	0	0
15	100 mgm. of NO_3 as calcium nitrate	0	(b)	0	0
16	150 mgm. of NO_3 as calcium nitrate	(b)	0	0	0

[a] Fungus contamination. [b] Plant died after few days' growth.

It will be seen that in a few instances where a high concentration of nitrates occurred the development of the seedlings subsequent to germination ceased. This condition may have been due to too high a concentration of soluble salts or to inferior seed. However, losses were not sufficiently serious materially to affect the outcome of the experiment.

In all cases the seedlings grown in agar without nitrate produced nodules when inoculated with *B. radicicola.* A few nodules appeared on seedlings in cultures containing the lowest concentration of all three nitrates. The number of nodules in these cases was less than in the control cultures. No nodules whatever developed in any concentration above 10 mgm. of nitrate in 100 c. c. of medium. Under normal conditions in test-tube cultures the nodules make their appearance at about 18 to 20 days after inoculation. The incubation of all cultures was extended 40 days after inoculation in order to make certain that no further nodule development would take place.

The nonproduction of nodules was not due to the failure of the inoculum. In all cases an excellent inoculum growth was obtained, especially in the case where nitrate was present in the medium. Indeed, it was so luxuriant that in many cases the organism grew in considerable quantity far down into the root zone. In many cases where nitrates were present the same pink coloration was produced that was discussed under another caption, on page 216.

As has been already stated, seedlings of varying ages were inoculated for the reason that it was thought that a more or less prolonged contact of the roots with the nitrate in the medium might serve as an index to

the time in the growth of the seedling when permanent resistance to attack of the organisms was established. The results obtained do not seem to indicate that seedling roots 18 to 20 days' old are any more resistant to the attack of the organisms than are those that are younger. Evidently if any reaction takes place between the nitrate and the plant root it occurs during the very early stages in the development of the plant.

These results seem to point to the conclusions that the nonformation of nodules in the presence of nitrates is due not to a weakening of the vitality of the organism, but to some reaction between the plant root and nitrate. One naturally queries whether the plant root cells are made more resistant to the bacteria seeking to gain entrance there or whether the naturally occurring carbohydrate food supply to be used by the organisms after gaining entrance is diminished by its conversion into protein material by the absorption of nitrate? Further studies were not made in an endeavor to solve this question.

INFLUENCE OF NITRATES IN SOIL ON ALFALFA NODULES AND ON THE REFORMATION OF NODULES

Additional studies were made with nitrates in relation to their influence on nodules already formed and on the redevelopment of nodules once removed from alfalfa plants. The experiments were carried out in an endeavor to determine whether nitrates prevented an increase in the number of nodules on plants possessing nodules and whether they prevented the reformation of nodules when removed. Experiments revealed clearly that removed nodules were replaced by new ones provided the plant was carefully replaced in the soil (soil with normal low nitrate content) and the proper amount of moisture maintained.

In these experiments 1-gallon earthenware jars were used. These were filled to within an inch of the top with 1,800 gm. of soil of a low nitrate content. Different amounts of the nitrates to be studied were added in the quantities indicated in Table XXXI. Concentrations of 100 and 300 mgm. of nitrate in 100 gm. of soil were also used, but the transplanted alfalfa seedlings were unable to withstand such excessive concentration, with the result that all died within a week or ten days after transplanting. Quadruplicate pots were prepared for each concentration of nitrate. The nitrates in solution were mixed with the proper amount of distilled water which, when added to the pots, brought the moisture content to approximately 20 per cent. The pots were then allowed to remain undisturbed for one day at room temperature to allow the water containing the nitrate to become well diffused throughout the soil mass. Into two pots of each set were transplanted young alfalfa plants from which the nodules had been removed. The two remaining pots contained transplanted alfalfa plants with the nodules left on and their location noted. The plants used in this experiment

were removed from an alfalfa plot, the soil of which was a sandy loam. Previous to transplanting the roots of the young plants were carefully washed in running water and immediately transplanted. The pots were kept well watered, and after two or three days they were removed to the greenhouse. Here they were watered when necessary. Transplantations were made on the 27th of June and the experiment terminated on the 3d of August. The plants were removed from the pots, the roots carefully washed and examined for the presence of nodules. The results are presented in Table XXXI.

TABLE XXXI.—*Influence of nitrates in soil on alfalfa nodules and on the reformation of nodules*

Pot No.	Nitrate in 100 gm. of dry soil.	Treatment of nodules.	Number of nodules—	
			At beginning.	At end.
A 1	None	Removed	0	3
A 2	do	do	0	4
A 3	do	Not removed	4	8
A 4	do	do	3	7
B 1	25 mgm. of NO_3 as potassium nitrate	Removed	0	0
B 2	do	do	0	(a)
B 3	do	Not removed	4	3
B 4	do	do	8	5
C 1	50 mgm. of NO_3 as potassium nitrate	Removed	0	0
C 2	do	do	0	0
C 3	do	Not removed	4	2
C 4	do	do	1	1
D 1	25 mgm. of NO_3 as sodium nitrate	Removed	0	0
D 2	do	do	0	0
D 3	do	Not removed	4	2
D 4	do	do	5	1
E 1	50 mgm. of NO_3 as sodium nitrate[b]	Removed	0	0
E 2	do	do	0	(a)
E 3	do	Not removed	2	1
E 4	do	do	6	3
F 1	25 mgm. of NO_3 as calcium nitrate	Removed	0	0
F 2	do	do	0	0
F 3	do	Not removed	2	2
F 4	do	do	4	3
G 1	50 mgm. of NO_3 as calcium nitrate	Removed	0	0
G 2	do	do	0	0
G 3	do	Not removed	4	3
G 4	do	do	2	1

[a] Plants died.

It will be seen in the control pots, where no nitrate was present (except the small amount normally present in the soil at the beginning of the experiment), that if the nodules were removed, new ones formed. The location of the nodules before their removal was noted, and the new ones were found to occupy the same place. However, when nitrates were added to the soil no new nodules were formed. This statement holds true for both concentrations of all three salts in all experiments.

Some interesting results were obtained where the nodules were not removed. In the control pots an increase in nodule formation took place. It can not be stated definitely whether the new nodules appeared as a result of inoculation from the soil or whether the organisms had already gained entrance to the roots before the plants were removed from the field soil previous to transplanting. Nevertheless, it is shown that the number of nodules increased as compared with the number present at the time of transplanting. But where nitrates were added a reduction in number occurred rather regularly throughout all the pots. In two instances the number remained constant, in 10 it was reduced, and in none was it increased. The calcium salt appeared to effect the least reduction in number of nodules. Conclusions concerning the comparative influence of the three salts in this regard can not be drawn because of the small number of determinations made. It is sufficient to note that nitrates present in amounts equal to 25 and 50 mgm. of nitrate in 100 gm. of soil did not permit an increase in number of nodules, but rather caused a decrease.

The conclusions drawn from the experiments relative to the influence of nitrates on nodule formation are: (a) the presence of nitrates is detrimental to the formation of nodules by alfalfa; (b) the nonformation of nodules is not due to a weakening of *B. radicicola* when grown in the presence of nitrates; (c) some reaction takes place between the nitrates and the plant root, thus preventing nodule formation; (d) nitrates in the soil prevent the re-formation of nodules once removed and also cause a decrease in the number of those already present.

SUMMARY

(1) Small quantities of potassium, sodium, and calcium nitrates caused a great increase in the number of Azotobacter in sterilized soil. Ammonium nitrate in the same quantities caused a less marked increase. Higher concentrations were not so favorable to the growth of the organisms.

(2) Potassium and sodium nitrates in the concentrations studied caused an increase in the amount of nitrogen assimilated by Azotobacter on agar films. Calcium nitrate in the same amounts brought about a decrease in the amount of nitrogen fixed to a point even below that representing the amount assimilated in the absence of nitrates. In soil cultures nitrates of sodium and calcium caused an increase in total nitrogen, which was more marked in the unsterilized cultures than in those cultures sterilized and inoculated with a pure culture of Azotobacter. However, the increase in total nitrogen is not commensurate with the increase in the number of Azotobacter noted under the same conditions.

(3) Under aerobic conditions Azotobacter in liquid cultures reduced nitrate to nitrite, but not to ammonia. More atmospheric nitrogen was assimilated in the presence of nitrate than in the absence of this salt.

(4) Pigmentation occurred when potassium and sodium nitrates, and especially calcium nitrate, were used with Azotobacter, the coloration increasing with the concentration of the salt. This effect was more marked in Azotobacter strains which produce little or no pigment in the absence of nitrates.

(5) All three nitrates studied caused an increase in the number and size of volutin bodies in Azotobacter cells. From all appearances these salts also tended to hasten the development of these bodies.

(6) The number of *Bacillus radicicola* in sterilized soil was increased by the addition of small quantities of potassium, sodium, ammonium, and calcium nitrates. This increase was not so marked as in the Azotobacter cultures. *B. radicicola* appeared to be much more resistant to higher concentrations of nitrates than Azotobacter.

(7) *B. radicicola* under aerobic conditions did not reduce nitrates in solution to nitrite, ammonia, or elemental nitrogen. The presence of nitrates did not materially influence the small amount of atmospheric nitrogen fixed under these conditions.

(8) When grown on agar films, *B. radicicola* fixed a small amount of nitrogen, varying from 0.15 to 0.43 mgm. of nitrogen in 100 c. c. of the medium. The addition of various amounts of potassium, sodium, and calcium nitrates increased to a slight extent the amount of nitrogen assimilated.

(9) In liquid cultures all three nitrates caused a large increase in the amount of gum obtained by precipitation with acetone.

(10) The presence of large amounts of potassium, sodium, and calcium nitrates proved detrimental to the formation of nodules on alfalfa. *B. radicicola* did not appear to lose its infecting power when grown on media containing varying amounts of sodium and calcium nitrates. Alfalfa seedlings grown in the presence of large amounts of nitrate did not produce nodules when inoculated with a viable culture of *B. radicicola*. Nitrates in soil cultures prevented the re-formation of nodules once removed and also caused a decrease in the number of nodules already present.

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It will be seen that all three nitrates exerted considerable influence on the formation of volutin bodies. Not only was the number of bodies increased, but also the size. The relative increase in size of the granules was much more marked than was the numerical increase. In Azotobacter cells grown on mannit agar containing no nitrate the number of volutin bodies in each cell averaged about two; in the presence of nitrate four to five volutin granules were found. The greatest increase in number, as well as size, occurred where the nitrate concentration was highest. With both strains sodium nitrate apparently caused the greatest increase. This was true in the lower as well as in the higher concentrations. The volutin bodies in strain B seemed to respond to the presence of nitrates more noticeably than did those of strain A, especially in the presence of potassium nitrate. It is evident that nitrates of potassium, sodium, and calcium cause an increase in the number and size of volutin bodies in Azotobacter cells.

Do these salts tend to hasten the appearance of these bodies, or do they at first retard their development? The following experiment was carried out in an endeavor to determine this point. Only sodium nitrate was used, since this particular salt proved most beneficial to the formation of volutin bodies. Agar slopes were prepared containing the different amounts of nitrate as indicated in Table XIX. The cultures were incubated at 28° C. and examined daily for the presence of volutin bodies. The methylene blue—1 per cent sulphuric acid—method of staining was employed. The results of the experiment are given in Table XIX.

TABLE XIX.—*Influence of sodium nitrate on the rate of formation of volutin bodies in Azotobacter*

Time.	Nitrate in 100 c. c. of medium.							
	Strain A.				Strain B.			
	0 Mgm.	25 Mgm.	100 Mgm.	300 Mgm.	0 Mgm.	25 Mgm.	100 Mgm.	300 Mgm.
Day.								
1	Absent	Absent	Doubtful.	Doubtful.	Absent	Absent	Doubtful.	Dbtful.
2	Present [a].	Present [a].	Present [a].	do. [b]	do	Present [a].	do. [b]	Do. [b].
3	do. [a]	do. [a]	do. [a]	do. [b]	Present [a].	do. [b]	do. [b]	Do. [b].
4	do. [a]	do. [a]	do. [b]	do. [b]	do. [a]	do. [b]	do. [b]	Do. [b].

[a] Representing an approximate average of two volutin bodies per cell.
[b] Representing an approximate average of four volutin bodies per cell.

A study of Table XIX shows that it is rather doubtful whether the nitrate present tended to hasten the appearance of the volutin bodies. No convincing evidence has been presented for or against this statement. No granules were seen in the first day's growth of strain A, although the next day they were present in all four cultures. In strain B more convincing proof is furnished that the sodium nitrate hastened the appearance of these reserve food substances. The volutin bodies were not present in the control and lowest nitrate concentration cultures the first day, but they were very noticeable in the culture containing the highest concentration of nitrate and doubtful in the remaining one. On the second day volutin bodies were present in all cultures grown on

nitrate media, while the control culture was still free from them. The third day showed the presence of volutin bodies in all four cultures. Strain B offers the better proof that sodium nitrate tends to hasten the appearance of volutin bodies in the cells of Azotobacter. Further experiments were not made in an endeavor to determine what influence nitrates might have on the cytology of the Azotobacter cell. The brief studies reported here were made in connection with the pigment formation experiments, but do not bear any particular relation to them. The increase in number and size of volutin bodies may bear some relation to the increased amount of nitrogen fixed or assimilated by Azotobacter in the presence of nitrates.

INFLUENCE OF NITRATES ON BACILLUS RADICICOLA

INFLUENCE OF NITRATES ON THE GROWTH AND REPRODUCTION OF BACILLUS RADICICOLA IN STERILIZED SOIL

One hundred and fifty gm. (dry weight) of the soil were weighed into 500-c. c. Erlenmeyer flasks and the nitrates added as indicated in Tables XX–XXII. Duplicate cultures for each amount of nitrate were prepared. One per cent of mannit (in 5 c. c. of distilled water) was also added. The flasks were kept at room temperature for one day and the contents then thoroughly mixed. The flasks were sterilized at 15 pounds' pressure for three hours. Upon cooling they were inoculated with 5 c. c. of a suspension of *Bacillus radicicola* in sterile distilled water. The number of bacteria in the inoculum was determined. The moisture content was then approximately 18 to 20 per cent. The flasks were incubated at 28° to 30° C. and mannit-agar plates poured at the end of one and two weeks. The results of these experiments are given in Tables XX, XXI, and XXII, in which each figure represents an average of duplicate plates.

TABLE XX.—*Influence of potassium nitrate on Bacillus radicicola in sterilized soil*

Culture No.	Treatment (nitrate in 100 gm. of dry soil).	Number of organisms in 1 gm. of dry soil.				
		At beginning.	After 1 week.	Relative.	After 2 weeks.	Relative.
	Mgm.			*Per cent.*		*Per cent.*
1	0	10, 670	680, 000	100	8, 015, 000	100
2	0	10, 670	825, 000		7, 000, 000	
3	10	10, 670	2, 195, 000	372	14, 600, 000	171
4	10	10, 670	3, 410, 000		11, 050, 000	
5	25	10, 670	3, 900, 000	517	15, 400, 000	201
6	25	10, 670	3, 885, 000		14, 800, 000	
7	50	10, 670	1, 555, 000	208	11, 500, 000	173
8	50	10, 670	1, 585, 000		14, 400, 000	
9	100	10, 670	445, 000	51	2, 680, 000	40
10	100	10, 670	320, 000		3, 290, 000	
11	150	10, 670	375, 000	46	560, 000	8. 9
12	150	10, 670	330, 000		790, 000	
13	200	10, 670	135, 000	20	90, 000	1. 2
14	200	10, 670	170, 000		(a)	
15	300	10, 670	45, 000	6. 3	25, 000	. 36
16	300	10, 670	50, 000		30, 000	

a Contamination.

TABLE XXI.—*Influence of sodium nitrate on Bacillus radicicola in sterilized soil*

Culture No.	Treatment (nitrate in 100 gm. of dry soil).	Number of organisms in 1 gm. of dry soil.				
		At beginning.	After 1 week.	Relative.	After 2 weeks.	Relative.
	Mgm.			*Per cent.*		*Per cent.*
1	0	15, 500	1, 500, 000	100	6, 750, 000	100
2	0	15, 500	1, 250, 000		5, 950, 000	
3	10	15, 500	2, 560, 000	201	10, 000, 000	177
4	10	15, 500	3, 000, 000		12, 500, 000	
5	25	15, 500	6, 150, 000	418	14, 650, 000	240
6	25	15, 500	5, 375, 000		15, 700, 000	
7	50	15, 500	4, 850, 000	378	(a)	134
8	50	15, 500	5, 570, 000		8, 500, 000	
9	100	15, 500	2, 000, 000	140	1, 520, 000	25
10	100	15, 500	1, 850, 000		1, 650, 000	
11	150	15, 500	1, 060, 000	69	850, 000	14
12	150	15, 500	835, 000		940, 000	
13	200	15, 500	760, 000	54	500, 000	8. 8
14	200	15, 500	725, 000		620, 000	
15	300	15, 500	250, 000	22	150, 000	2. 8
16	300	15, 500	365, 000		210, 000	

a Contamination.

TABLE XXII.—*Influence of calcium nitrate on Bacillus radicicola in sterilized soil*

Culture No.	Treatment (nitrate in 100 gm. of dry soil).	Number of organisms in 1 gm. of dry soil.				
		At beginning.	After 1 week.	Relative.	After 2 weeks.	Relative.
	Mgm.			*Per cent.*		*Per cent.*
1	0	10, 000	960, 000	100	4, 675, 000	100
2	0	10, 000	850, 000		4, 590, 000	
3	10	10, 000	3, 650, 000	419	6, 000, 000	124
4	10	10, 000	3, 940, 000		5, 450, 000	
5	25	10, 000	5, 500, 000	674	10, 650, 000	274
6	25	10, 000	6, 700, 000		14, 700, 000	
7	50	10, 000	4, 000, 000	414	9, 350, 000	195
8	50	10, 000	3, 500, 000		8, 670, 000	
9	100	10, 000	1, 200, 000	180	1, 500, 000	35
10	100	10, 000	2, 050, 000		1, 750, 000	
11	150	10, 000	865, 000	106	765, 000	17
12	150	10, 000	1, 050, 000		800, 000	
13	200	10, 000	375, 000	35	350, 000	7. 0
14	200	10, 000	260, 000		300, 000	
15	300	10, 000	35, 000	4. 5	25, 000	. 70
16	300	10, 000	47, 000		40, 000	

An inspection of all three tables reveals two marked differences from the results obtained in similar work with Azotobacter. First, it will be noted that nitrates do not appear to exert such a marked stimulating effect with *B. radicicola* as with Azotobacter. The numerical increase due to the presence of the nitrate is clearly shown in the percentage columns. Second, it will be noted that *B. radicicola* does not seem to be so sensitive to higher concentrations of nitrates as does Azotobacter. In all instances at concentrations equivalent to 300 mgm. of nitrate in

100 gm. of soil the legume organisms were still alive, although present in numbers far below those of the control cultures. In all Azotobacter cultures no organisms survived this concentration.

No one nitrate produced an excessive stimulation in comparison with the others. The calcium salt present as 150 mgm. of nitrate in 100 gm. of soil at the end of the first week gave the greatest stimulation for concentrations of that amount. However, at the end of the second week this concentration had caused a marked decrease in the number of organisms. In the case of all three nitrates the concentration representing 25 mgm. of nitrate in 100 gm. of soil produced the greatest stimulation. This resulting stimulation also held true throughout the second week. The decrease in number below those of the control cultures, due to increasing concentrations of nitrate, began first in the presence of potassium nitrate at 100 mgm. of nitrate per 100 gm. of soil, then with sodium nitrate at 150 mgm., and lastly with calcium nitrate at 200 mgm. But the number of organisms present in the soil cultures containing sodium nitrate in amounts equivalent to 100 mgm. and calcium nitrate at 100 mgm. at the end of the second week was below those of the control cultures.

It therefore appears from these results that small amounts of potassium, sodium, and calcium nitrate stimulate the reproductive activity of *B. radicicola*. Concentrations of nitrates greater than those amounts which produced maximum stimulation cause a decrease in the number of organisms. The highest concentration of nitrate studied did not entirely prevent the growth of the bacteria, but it reduced the number of organisms far below those contained in control cultures where no nitrates were added.

Ammonium nitrate was also employed. The soil cultures were prepared as already described and inoculated with *B. radicicola*. The cultures were incubated at 28° to 30° C. and counts were made at the end of one and two weeks' time. The results of the study with ammonium nitrate are given in Table XXIII.

TABLE XXIII.—*Influence of ammonium nitrate on Bacillus radicicola in sterilized soil*

Culture No.	Treatment (nitrate in 100 gm. of dry soil).	Number of organisms in 1 gm. of dry soil.				
		At beginning.	After 1 week.	Relative.	After 2 weeks.	Relative.
	Mgm.			*Per cent.*		*Per cent.*
1	0	10,000	850,000	100	1,365,000	100
2	0	10,000	765,000		1,400,000	
3	25	10,000	2,500,000	343	5,060,000	338
4	25	10,000	3,050,000		4,320,000	
5	100	10,000	1,350,000	148	1,030,000	71
6	100	10,000	1,050,000		950,000	
7	200	10,000	700,000	84	635,000	45
8	200	10,000	655,000		605,000	

From the results as a whole it appears that it is the nitrate radical and not the combined salt which causes the increase in the number of *B. radicicola* when small amounts of nitrates are present. A stimulation occurred, resulting in an increase in number which is quite comparable to that obtained with potassium, sodium, and calcium nitrates. The highest concentration of ammonium nitrate used did not appear to have such an inhibiting effect as did the corresponding concentrations of the three other salts.

Throughout the work with *B. radicicola* in sterilized soil comparatively low numbers of these organisms were found. Whether or not this depression was due to toxic substances formed as a result of sterilization can not be stated. If this decrease in numbers as a result of the presence of toxic substances is true, it is very evident that the detrimental effect had not become materially lessened at the end of the incubation period. However, in any event the validity of the outcome is not impaired, since comparative and not absolute data are of importance and since in all probability the same conditions obtained throughout the cultures.

It seems certain from the results of these studies on the effect of potassium, sodium, calcium, and ammonium nitrates on the growth of *B. radicicola* in sterilized soil that small amounts of nitrate stimulate the growth of the organisms. It is also shown that *B. radicicola* is much more resistant than Azotobacter to higher concentrations of potassium, sodium, calcium, and ammonium nitrates.

INFLUENCE OF BACILLUS RADICICOLA ON NITRATES IN SOLUTION

The series of soil culture experiments just discussed served to give an idea concerning the effect of nitrates on the legume organism. It was found that in small amounts nitrates stimulated the bacteria to increased reproduction. But no study was made as to the effect of *Bacillus radicicola* on the nitrate. Does the organism break up the nitrate, reducing it to nitrite or ammonia? Does it cause any loss in nitrate when grown in a solution containing that salt? Beijerinck (*2, p. 762*) as a result of physiological experiments with *B. radicicola*, states that the organism does not reduce nitrate. Prucha (*41*) also states that *B. radicicola* does not reduce nitrates. However, Zipfel (*49*) found that *B. radicicola* will reduce nitrates to nitrites but not to ammonia.

The following experiments, somewhat similar to those already cited in relation to Azotobacter, were carried out in an endeavor to answer these questions.

To twenty 500-c. c. Erlenmeyer flasks containing 200 c. c. of mannit solution, potassium, sodium, calcium, and ammonium nitrates were added as indicated in Tables XXIV, XXV, and XXVI. Quadruplicate flasks were prepared for each concentration of nitrate and for the control cultures without nitrate. The flasks and contents were sterilized at

10 pounds' pressure for 30 minutes. After cooling, two of each set of four flasks were inoculated with 5 c. c. of a suspension of *B. radicicola* in sterile distilled water. The remaining two flasks of each set (uninoculated) served as controls. The flasks were incubated at 28° C. for 21 days. The total weight of the flasks was maintained throughout the incubation period by the addition of sterile distilled water from time to time. At the expiration of the period of incubation the nitrate, ammonia, and total nitrogen contents were determined as given under "Methods used in experiments." The contents of the duplicate inoculated flasks were poured together and 50 c. c. samples drawn for analysis. The same procedure was followed in the case of the uninoculated flasks. The results are given in Tables XXIV, XXV, and XXVI.

TABLE XXIV.—*Influence of Bacillus radicicola on nitrates in solution giving the quantity of nitrate lost*

Culture No.	Treatment (nitrate in 100 c. c. of medium).	Nitrate in 100 c. c. of medium.				Nitrate lost.
		Uninoculated.		Inoculated.		
		Found.	Average.	Found.	Average.	
		Mgm.	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*
1	None	0.00	0.00	0.00	0.00	0.00
2	.....do	.00		.00		
3	150 mgm. of NO_3 as potassium nitrate	151.4	151.2	117.0	117.0	−34.2
4	.....do	151.0		117.0		
5	150 mgm. of NO_3 as sodium nitrate	148.8	148.8	114.4	114.2	−34.6
6	.....do	148.8		114.0		
7	150 mgm. of NO_3 as calcium nitrate	154.8	155.2	76.6	76.7	−78.5
8	.....do	155.6		76.8		
9	150 mgm. of NO_3 as ammonium nitrate	151.4	151.5	142.6	142.6	− 8.9
10	.....do	151.6		142.6		

TABLE XXV.—*Influence of Bacillus radicicola on nitrates in solution giving the quantity of nitrogen as ammonia formed*

Culture No.	Treatment (nitrate in 100 c. c. of medium).	Nitrogen as ammonia in 100 c. c. of medium.				Nitrogen as ammonia formed.
		Uninoculated.		Inoculated.		
		Found.	Average.	Found.	Average.	
		Mgm.	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*
1	None	0.20	0.15	0.10	0.15	0.00
2	.....do	.10		.20		
3	150 mgm. of NO_3 as potassium nitrate	.10	.15	.00	.05	−.10
4	.....do	.20		.10		
5	150 mgm. of NO_3 as sodium nitrate	.20	.20	.20	.25	+.05
6	.....do	.20		.30		
7	150 mgm. of NO_3 as calcium nitrate	.40	.35	.30	.20	−.15
8	.....do	.30		.10		
9	150 mgm. of NO_3 as ammonium nitrate	13.90	13.92	13.80	13.82	+.10
10	.....do	13.95		13.85		

TABLE XXVI.—*Influence of Bacillus radicicola on nitrates in solution giving the quantity of nitrogen fixed*

Culture No.	Treatment (nitrate in 100 c. c. of medium).	Total nitrogen in 100 c. c. of medium. Uninoculated. Found.	Uninoculated. Average.	Inoculated. Found.	Inoculated. Average.	Nitrogen fixed.
		Mgm.	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*
1	None	2. 40	2. 45	3. 30	3. 40	0. 95
2	....do	2. 50		3. 50		
3	150 mgm. of NO_3 potassium nitrate	18. 00	17. 95	18. 70	18. 85	. 90
4	....do	17. 90		19. 00		
5	150 mgm. of NO_3 sodium nitrate	16. 80	16. 90	19. 30	19. 25	2. 35
6	....do	17. 00		19. 20		
7	150 mgm. of NO_3 calcium nitrate	14. 00	13. 90	14. 60	14. 65	. 75
8	....do	13. 80		14. 70		
9	150 mgm. of NO_3 ammonium nitrate	40. 50	40. 85	41. 30	41. 50	. 65
10	....do	41. 20		41. 70		

The data in Table XXIV show that a rather large reduction in the total nitrate content took place. This reduction varied rather markedly among the four different nitrates studied. The greatest reduction occurred where calcium nitrate was used. Potassium and sodium were next in order; the loss was almost the same for both salts. Ammonium nitrate was last with but a very small comparative reduction in total nitrate.

The question arises as to whether the nitrate is reduced to nitrite, ammonia, or elemental nitrogen or whether the reduction in amount is due to a natural assimilation of the nitrate by the organisms. The first possibility was precluded when qualitative tests for nitrites were made and none found. Table XXV reveals the fact that no ammonia was produced. Table XXVI shows no loss in total nitrogen. Therefore it seems obvious that reduction in total amount of nitrate present is brought about by the assimilation of those compounds by the organisms.

An inspection of Table XXVI, which gives the results of the total nitrogen determinations, shows that a slight fixation of atmospheric nitrogen took place. This fixation is entirely possible, as will be shown later when the influence of nitrates on the fixation of nitrogen is taken up. In the presence of potassium, sodium, and ammonium nitrates the amount of nitrogen assimilated is somewhat decreased. But in the case of sodium nitrate a large increase in the amount of total nitrogen seems to have taken place. This is interesting in the light of results to be presented later.

From the results of the work on the effect of *B. radicicola* on nitrates it may be concluded that the organisms do not reduce the nitrates to nitrite or ammonia or elemental nitrogen under aerobic conditions.

INFLUENCE OF NITRATES ON THE FIXATION OF ATMOSPHERIC NITROGEN BY BACILLUS RADICICOLA

The ability of *B. radicicola* to fix atmospheric nitrogen in the absence of the host plant has been studied by numerous investigators. From the results of their work it seems fairly probable that the legume organism can fix nitrogen to a slight extent when growing in a nonsymbiotic state. Beijerinck (*3*) was one of the earliest to make a study of the possible fixation of atmospheric nitrogen by *B. radicicola* under these conditions. He found that a small quantity, 0.91 to 1.82 mgm. of nitrogen was fixed per 100 c. c. of the medium. Prasmowski (*39, p. 55*) and Berthelot (*6*) concluded as a result of their experiments that when the organism was grown outside the host plant the gain in nitrogen was small. The greatest gain in nitrogen was found by Mazé (*32*) who reported an increase of 23.4 mgm. of nitrogen per 100 c. c. of the medium in 16 days. Lewis and Nicholson (*30*) found by incubating the cultures for a considerable length of time that a large increase in fixation occurred. Bottomley (*8*) found that a pure culture of *B. radicicola* fixed approximately 1 mgm. of nitrogen in 15 days. Fred (*17*) made a study of the possible fixation of nitrogen by the legume organism and found that it fixed approximately 1.2 mgm. of nitrogen in 100 c. c. of the medium. He found that on agar films a greater fixation occurred than when the organisms were grown in a liquid medium.

A few investigators, however, found that no increase in nitrogen occurred when *B. radicicola* was grown outside the host plant. Frank (*16*) states that in a nitrogen-free medium the legume organisms did not fix enough nitrogen to be accurately measured. Immendorf (*25*) also found no increase in nitrogen when pure cultures of *B. radicicola* were grown in soil containing a nitrogen-free solution.

It will be seen that the majority of investigators, especially the more recent ones, found that a slight amount of atmospheric nitrogen was fixed or assimilated by *B. radicicola* when grown outside the host plant and on a medium suitable for its development.

It has already been shown that nitrates cause an increase in the number of *B. radicicola* when grown in pure culture in sterilized soil. Does such an increase in the number of organisms necessarily mean an increased fixation of nitrogen? Three experiments using agar films were carried out in order to determine this point. Erlenmeyer flasks of 1-liter capacity containing 100 c. c. of mannit agar were used. Before the medium solidified, the nitrates were added in the proportions indicated in Table XXVII. Six flasks for each different quantity of nitrate were prepared, except in one case, as shown in Experiment II. The flasks were plugged with nonabsorbent cotton and sterilized at 10 pounds' pressure for 30 minutes. After cooling, three of each set were inoculated with 5 c. c. of a suspension of *B. radicicola* in sterile distilled water. The organisms had been growing on mannit agar at 28° C. for six days. The flasks in Experiments I and III (Table XXVII) were incubated at

28° C. for three weeks and those in Experiment II for two weeks. The moisture lost by evaporation in both inoculated and uninoculated flasks was replaced from time to time by the addition of sterile distilled water. At the expiration of the incubation period the total nitrogen was determined as given under "Methods used in experiments." The results of the experiments are given in Table XXVII.

An inspection of the data reveals the fact that *B. radicicola* in pure culture fixed a small amount of nitrogen when growing in a nonsymbiotic state with no nitrate present. In the presence of nitrates there was an increased fixation. Although the increase in total nitrogen is small, because of the number of determinations made, it may be considered as positive. The potassium and sodium salts seemed to be more effective than the calcium nitrate, with one exception (Table XXVII, Experiment I). It will be remembered that the latter salt appeared to depress nitrogen fixation by Azotobacter and the two former somewhat to favor it (p. 194–195).

TABLE XXVII.—*Influence of nitrates on the fixation of nitrogen by Bacillus radicicola, giving the increase in nitrogen*

EXPERIMENT I

Culture No.	Treatment (nitrate in 100 c. c. of medium.)	Total nitrogen in 100 c. c. of medium. Uninoculated. Found.	Total nitrogen in 100 c. c. of medium. Uninoculated. Average.	Total nitrogen in 100 c. c. of medium. Inoculated. Found.	Total nitrogen in 100 c. c. of medium. Inoculated. Average.	Nitrogen increase.
		Mgm.	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*
1	None	4. 5		4. 7		
2	.....do	4. 4	4. 45	4. 6	4. 60	0. 15
3	.....do	4. 4		4. 5		
4	75 mgm. of NO_3 as sodium nitrate	8. 7		11. 9		
5	.....do	8. 7	8. 70	11. 8	11. 75	3. 05
6	.....do	8. 6		11. 6		
7	150 mgm. of NO_3 as sodium nitrate	12. 5		14. 9		
8	.....do	12. 7	12. 60	14. 6	14. 70	2. 10
9	.....do	....		14. 7		
10	75 mgm. of NO_3 as calcium nitrate	8. 8		12. 3		
11	.....do	8. 9	8. 90	12. 8	12. 40	3. 50
12	.....do	9. 0		12. 1		
13	150 mgm. of NO_3 as calcium nitrate	13. 3		14. 5		
14	.....do	13. 1	13. 20	14. 0	14. 10	0. 90
15	.....do	13. 2		13. 8		

EXPERIMENT II

Culture No.	Treatment (nitrate in 100 c. c. of medium.)	Uninoculated. Found.	Uninoculated. Average.	Inoculated. Found.	Inoculated. Average.	Nitrogen increase.
1	None	4. 90	4. 90	5. 10	5. 075	0. 175
2	.....do	4. 90		5. 05		
3	75 mgm. of NO_3 as sodium nitrate	8. 70	8. 60	9. 20	9. 50	0. 90
4	.....do	8. 50		9. 80		
5	150 mgm. of NO_3 as sodium nitrate	13. 30	13. 15	14. 20	14. 35	1. 20
6	.....do	13. 00		14. 50		
7	75 mgm. of NO_3 as calcium nitrate	11. 15	11. 125	11. 60	11. 65	0. 525
8	.....do	11. 10		11. 70		
9	150 mgm. of NO_3 as calcium nitrate	14. 70	14. 70	15. 40	15. 25	0. 550
10	.....do	(a)		15. 10		

[a] Lost by breakage during sterilization.

TABLE XXVII.—*Influence of nitrates on the fixation of nitrogen by Bacillus radicicola, giving the increase in nitrogen*—Continued

EXPERIMENT III

Culture No.	Treatment (nitrate in 100 c. c. of medium):	Total nitrogen in 100 c. c. of medium. Uninoculated. Found.	Uninoculated. Average.	Inoculated. Found.	Inoculated. Average.	Nitrogen increase.
		Mgm.	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*
1	None	5.10		5.50		
2	.....do	5.10	5.07	5.40	5.50	0.43
3	.....do	5.00		5.45		
4	75 mgm. of NO_3 as potassium nitrate	9.35		10.85		
5	.....do	9.50	9.37	10.90	10.90	1.53
6	.....do	9.25		10.95		
7	150 mgm. of NO_3 as potassium nitrate	14.50		15.65		
8	.....do	14.20	14.28	15.30	15.45	1.17
9	.....do	14.15		15.40		
10	75 mgm. of NO_3 as sodium nitrate	8.50		9.85		
11	.....do	8.30	8.38	9.90	9.83	1.45
12	.....do	8.35		9.70		
13	150 mgm. of NO_3 as sodium nitrate	12.35		12.95		
14	.....do	12.40	12.33	13.10	13.03	0.70
15	.....do	12.20		13.05		
16	75 mgm. of NO_3 as calcium nitrate	8.95		9.85		
17	.....do	9.10	9.01	9.90	9.93	0.92
18	.....do	9.00		10.05		
19	150 mgm. of NO_3 as calcium nitrate	13.90		14.40		
20	.....do	13.80	13.80	14.50	14.42	0.62
21	.....do	13.70		14.35		

It has been shown that, when nitrates are added in varying quantities to sterilized soil, the number of *B. radicicola* are increased. Provided the the organism can fix a small amount of nitrogen in the absence of nitrate nitrogen, is it not possible that this increase in nitrogen fixation may be due merely to the increase in the number of cells? It seems that this is true according to the results in Table XXVII. It appears probable that the increase in nitrogen fixed in the presence of nitrates is very likely because of an increase in the number of bacterial cells and not to any physiological change brought about in the organism itself.

There was a marked increase in bacterial growth on the media containing the nitrate compared with the same media free from nitrate. The growth on the latter medium exhibited a normal, whitish watery appearance, characteristic of this organism. On the cultures containing nitrates a much more profuse growth occurred. In many instances a pinkish tint was observed. This pigment production was especially marked in the case of the culture containing the sodium salt. After the first experiment had been completed, it was thought that possibly this pigmentation was due to an impurity in the culture. Therefore the two remaining experiments were made, using a subculture from the original.

This culture was plated three times, each plating being made from a well-isolated colony. The final subculture was taken from a similar well-isolated colony. However, pigment formation in the presence of nitrate persisted in the two final experiments, showing clearly that some reaction took place between the nitrate and the organism grown on the medium. It is of interest to note that the pigment formation in the presence of nitrate was observed in later work where the influence of nitrates on nodule formation was investigated. Prucha (*41*) found that on agar slopes of medium containing 0.5 per cent of potassium or calcium nitrate, the growth of *B. radicicola* became opaque and that an iridescent tint was produced.

Although the results of these experiments may vary somewhat among themselves, taken as a whole it appears evident that B. *radicicola* may fix a small amount of atmospheric nitrogen when grown without the host plant and on a suitable medium. The addition of various amounts of nitrates as indicated increased somewhat the amount of nitrogen assimilated by *B. radicicola*.

INFLUENCE OF NITRATES ON THE PRODUCTION OF GUM BY BACILLUS RADICICOLA

Since nitrates, especially in smaller amounts, cause an increase in the number of *B. radicicola* in pure culture, it was thought advisable to determine what influence these salts have on the production of gum. In culture media favorable to the growth of *B. radicicola* these bacteria will produce a gelatinous substance which is readily precipitated with 95 per cent alcohol or acetone. Upon the addition of either of these precipitants a fairly heavy, water-white, frothy gelatinous mass is formed which soon rises to the surface of the liquid. Upon standing, this mass contracts somewhat, and portions of it may fall to the bottom of the liquid from which it has been precipitated.

Chemical analyses, according to Buchanan (*10*), have shown that this gum is a carbohydrate. Upon hydrolysis with 2 per cent sulphuric acid and 15 pounds' pressure for one hour, Fehling's solution is reduced, showing the presence of a sugar. The gum does not give proteid reactions with the Millon, biuret, or xanthoproteic tests. Hence, the gum is not protein in character; nor does it contain nitrogen in the combined form. Clearly it is a nonnitrogenous body.

In the experiment undertaken to determine whether nitrates influence the formation of gum only relative differences are noted. No attempt was made to obtain quantitative results.

Erlenmeyer flasks of 1-liter capacity containing 200 c. c. of mannit solution were used. The cultures contained various quantities of nitrate as indicated in Table XXVIII. Triplicate flasks for each amount of nitrate were prepared. In this table these three flasks are represented as "a," "b," and "c." After sterilization at 15 pounds' pressure for 25 minutes the flasks were cooled and inoculated with 5 c. c. of a suspension

of *B. radicicola* in sterile distilled water. The cultures were then incubated at room temperature (approximately 25° C.) for eight weeks.

At the expiration of the incubation period the contents of the flasks were poured into hydrometer cylinders of equal depth and diameter. One hundred and fifty c. c. of acetone were added to precipitate the gum. After careful shaking, the cylinders were covered with inverted petri dishes to prevent evaporation. At the end of 24 hours the amount of gum precipitated was observed. The relative amounts are recorded in Table XXVIII.

TABLE XXVIII.—*Influence of nitrates on the production of gum by Bacillus radicicola*

Culture No.	Treatment (nitrate in 100 c. c. of medium).	Relative production of gum—precipitated by acetone.		
		Flask a.	Flask b.	Flask c.
1	None	Large	Large	Large.
2	75 mgm. of NO_3 as potassium nitrate	Very large	Very large	Very large.
3	450 mgm. of NO_3 as potassium nitrate	Large	Large	Large.
4	75 mgm. of NO_3 as sodium nitrate	Very large	...do	Very large.
5	450 mgm. of NO_3 as sodium nitrate	Large	...do	Large.
6	75 mgm. of NO_3 as calcium nitrate	...do	...do	Do.
7	450 mgm. of NO_3 as calcium nitrate	...do	Considerable.	Considerable.

From the results it is certain that the nitrates, especially in the smaller of the two concentrates, caused a very considerable increase in the amount of gum produced by *B. radicicola.* The nitrates of potassium and sodium caused a production of more gum than did the calcium salt. It will be remembered that in the experiments where the influence of nitrates on the fixation of atmospheric nitrogen by *B. radicicola* was studied, less nitrogen was fixed in the presence of calcium nitrate than in the presence of the other two salts. Here again the greater stimulative action of potassium and sodium nitrates is emphasized.

Buchanan in his investigations on the formation of gum by *B. radicicola* has found that varying amounts of potassium nitrate in a 2 per cent saccharose solution or in a 2 per cent saccharose-clover-extract solution caused a slight increase in growth and in gum production.

It seems probable that the increased gum production in the nitrate cultures is caused not only by an increase in bacterial cells but also perhaps by an increased stimulation in the formation of gum by the cells themselves. The relative increase in the amount of gum produced in the presence of nitrates seems to be greater than the actual increase in number of organisms brought about by the stimulating effect of the nitrate. In the latter instance this stimulating effect has been determined in soil cultures only and so a fair basis of comparison can not be

found. Had the influence of nitrates on the growth and reproduction of *B. radicicola* been determined in liquid culture, as well as in soil cultures, then a comparison could have been made. Furthermore, the divergencies in the time element, eight weeks' incubation in the liquid cultures and three weeks in the soil cultures, are such as to render futile any attempt at correlation. It may be that the large formation of gum was due to the prolonged incubation. A shorter period of three weeks undoubtedly would show a relatively smaller amount of gum produced as a result of the presence of the nitrate.

However, from the results of the experiment it is certain that potassium, sodium, and calcium nitrate influence the formation of gum by *B. radicicola*. The three nitrates studied caused a large increase in the amount of gum obtained by precipitation with acetone. Calcium nitrate caused the least stimulation, but the difference was not large.

INFLUENCE OF NITRATES ON NODULE FORMATION

The results of numerous investigations have shown that nitrates retard and oftentimes entirely prevent the formation of nodules on leguminous plants when grown in soil or liquid cultures. Vines (*45*), working with the horse bean, found that the use of large amounts of nitrate in the form of potassium nitrate retarded nodule formation. He concluded that a decrease in the amount of nitrates meant an increase in the number of nodules. Woods (*48*) found that leguminous plants assimilated more nitrogen when they were grown in the absence of potassium and calcium nitrate than when thus supplied. His results seem to indicate that nodule development was retarded somewhat by these salts. Similar results were obtained by Frank (*16*). Nobbe and Richter (*37*) in 1902 grew soybeans in a rich garden soil and found upon inoculation that a gain of 74.7 per cent of nitrogen occurred. However, upon the addition of nitrates this gain was considerably reduced, the extent of the reduction corresponding to the amount of nitrate added. About this same time, Wohltmann and Bergené (*47*) using many different types of soils, found that nodules were not formed on the roots of peas when ammonium nitrate was added. Creydt (*12*) found that sodium nitrate retarded nodule formation on yellow lupines when these legumes were grown in soil. Fred and Graul (*18*) found that very small amounts of nitrates did not appreciably decrease nodule formation, but that larger amounts proved detrimental and finally prohibited entirely the development of nodules.

The presence of nitrates in culture solutions has also been found to reduce and oftentimes to inhibit the formation of nodules on leguminous plants. Marchal (*31*) concluded that alkaline nitrates in concentrations of 1 to 10,000 in liquid cultures prevented the formation of nodules on peas. Flamand (*15*) grew vetch and beans in a nutrient solution and

found that nitrates in the following amounts prevented nodule formations: potassium nitrate, 1 to 10,000, sodium nitrate 1 to 2,000, ammonium nitrate 1 to 2,000, and calcium nitrate 1 to 2,000 and 1 to 10.000. Hiltner's (*24*) experiments showed that 5 mgm. of nitrogen as potassium nitrate per liter prevented nodule formation on peas.

In contrast to these experiments Bässler (*1*) claimed that results obtained from his work indicated that no effect was noticed by adding nitrates to lupines growing in quartz sand.

The question naturally arises whether this condition is due to the weakening of the organism brought about by growth in a nitrated environment and to a consequent impairment or entire loss of its infecting power, or whether it is caused by some interreaction between the salt and the plant root, tending to increase the latter's resistance to the attack of this particular organism.

INFLUENCE OF NITRATES ON THE INFECTING POWER OF BACILLUS RADICICOLA

Some investigations have been carried out to determine what effect nitrates have on the legume organisms themselves. Wilson (*46*) showed that although nitrates inhibit the formation of nodules, the organisms capable of producing nodules did not lose their vitality or nodule-producing power when grown in the presence of nitrates. The results of Prucha (*41*) are in accord with those of Wilson. He found that *B. radicicola* does not seem to lose its infecting power when grown on media containing nitrate. During the course of his work he found that potassium and sodium nitrates inhibited the formation of nodules. Further evidence that the organisms appear to retain their vitality in the presence of nitrates is produced by the results of Mazé (*33, p. 15–17*), who showed that legume bacteria were able to fix a slight amount of nitrogen when grown in a soil extract solution containing 1 per cent sodium nitrate. Herke (*22*) states that potassium nitrate favors the growth of nodule bacteria.

However, other investigators state that nitrates have a harmful effect on *B. radicicola*. Laurent (*29, p. 134*) found that legume organisms failed to grow in a pea or lupine decoction containing nitrate in the form of potassium and sodium salts in amounts equivalent to 1 to 500 and 1 to 1,000. Moore (*35*) in his experiments demonstrated that nitrates at 1 to 10,000 were sufficient to prevent nodule formation. He states that *B. radicicola* loses its power of infection when grown in a medium containing nitrates.

From the results cited it can be seen that there is some disagreement as to the influence exerted by nitrates on *B. radicicola*. In some cases the organism seems to retain its vitality in the presence of nitrates, while in others it appears to have become weakened. It must be ad-

mitted, however, that the evidence seems to favor the former contention—namely, that nitrates do not cause the bacteria to lose their nodule-producing power.

In order to determine whether or not nitrates weaken these organisms, the following experiments were made: Slopes of mannit agar (in test tubes) containing various amounts of sodium and calcium nitrates as indicated in Table XXIX were inoculated with *B. radicicola*. These cultures were incubated at 28° C. for one week, when transfers were made to fresh nitrate media and incubated at 28° C. for another week. At the expiration of this time, three 4-day-old seedlings of alfalfa were inoculated with three drops of a suspension of the organism in 5 c. c. of sterile distilled water. The same slope cultures were incubated at 28° C. and used for all subsequent inoculations in this experiment. The inoculated seedlings were placed in the greenhouse under cheesecloth covering. The temperature here during the daytime averaged approximately 30° C. The seedlings were examined for the first appearance of nodules and in no case did they appear before 18 to 20 days. A total count of nodules on all plants was made at the end of 45 days. Three subsequent inoculations were made under the same conditions. In this way organisms in contact with nitrate for varying lengths of time could be used. The results of the inoculation experiments are given in Table XXIX.

TABLE XXIX.—*Influence of nitrates on the infecting power of Bacillus radicicola*

Culture No.	Treatment (nitrate in 100 c. c. of medium).	Number of nodules after 45 days.			
		Inoculated June 3.	Inoculated June 15.	Inoculated July 11.	Inoculated July 17.
1	None	5	4	7	3
2	15 mgm. of NO_3 as sodium nitrate	5	4	5	5
3	37 mgm. of NO_3 as sodium nitrate	7	6	15	5
4	75 mgm. of NO_3 as sodium nitrate	4	5	3	11
5	150 mgm. of NO_3 as sodium nitrate	5	8	4	8
6	225 mgm. of NO_3 as sodium nitrate	6	5	5	7
7	450 mgm. of NO_3 as sodium nitrate	2	4	6	3
8	None	7	8	7	8
9	15 mgm. of NO_3 as calcium nitrate	4	4	9	4
10	37 mgm. of NO_3 as calcium nitrate	9	4	8	6
11	75 mgm. of NO_3 as calcium nitrate	5	5	11	8
12	150 mgm. of NO_3 as calcium nitrate	7	5	9	9
13	225 mgm. of NO_3 as calcium nitrate	5	7	4	3
14	450 mgm. of NO_3 as calcium nitrate	6	8	6	3
15	Uninoculated	0	0	0	0
16	.....do	0	0	0[1]	0

From the results given in Table XXIX it is very evident that under the conditions of the experiment the legume organisms did not lose their power of producing nodules when grown on a medium containing

varying amounts of sodium and calcium nitrates. The numbers of nodules produced on the alfalfa plants by organisms grown on media containing nitrate do not vary widely from those on the plants inoculated with organisms grown on media containing no nitrate. Not only did the organisms fail to lose their nodule-producing power, but from all appearances their infecting power did not seem to be materially weakened.

It therefore seems apparent that an explanation for the failure of nodules to develop on leguminous plants in the presence of nitrates is not found in the theory that the organisms producing these nodules are weakened when grown in the presence of nitrates.

INFLUENCE OF NITRATES ON ALFALFA ROOTS AND NODULE FORMATION

The next step taken would naturally be in the direction of a study of the influence of the nitrates on the plant roots themselves in order to determine whether or not they thus are made more resistant to the attack of these organisms.

A review of the literature shows that almost nothing has been done touching this phase of the question. Wilson (*46*), studying the effect of certain salts on nodule production, states that possibly the salt has some effect on the root, making it resistant to the attack of the organism. Mazé (*33, p. 15–17*), who also concluded that nitrates did not cause *B. radicicola* to lose its infecting power, says that nodules do not develop on roots of legumes when nitrates are present because the carbohydrate in the roots is changed into protein material by the absorption of the nitrate.

Alfalfa seedlings (*Medicago sativa*) growing in soft agar containing potassium, sodium, and calcium nitrates, as indicated in Table XXX, were used in this study. Quadruplicate tubes were prepared for each amount of nitrate. The higher concentrations of the nitrate were not used, since it was found that germination and subsequent growth were considerably impaired in the presence of such large amounts. The tubes with the mannit agar and nitrate were sterilized at 15 pounds' pressure for 30 minutes. These were cooled and sterilized alfalfa seeds planted as given under "Methods used in experiments." The tubes were then placed in the greenhouse under cheesecloth covering and the seeds allowed to germinate. Germination took place in all instances, although it was retarded somewhat by the presence of the nitrate. At the end of five days the first tube of each set was inoculated with three drops of a suspension of *B. radicicola* in sterile distilled water. Subsequent inoculations were made as indicated in Table XXX. These were made at different intervals in order to allow the roots of the seedlings to remain for a longer time in contact with the media. It was hoped that in this way an idea might be obtained as to the time when the root first became resistant. The results are given in Table XXX.

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NEW-PLACE EFFECT IN MAIZE

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INTRODUCTION

Widely divergent opinions have been expressed regarding the advisability of transferring seed from one region to another. With maize (*Zea mays*) the transfer of seed is generally held to be disadvantageous. Numerous experiments have shown that when seed of the same original variety, but grown at two places, is planted side by side at one of the places the results are in favor of the local seed.

The relative superiority of the locally selected seed has been so pronounced that the securing of seed from distant localities, except where grown for forage, has been discouraged. The natural result is to confine the utilization of carefully selected strains to the localities where the breeding is done. Caution in transferring seed is certainly desirable, but the results of the experiments here reported indicate that two opposing factors are involved, the relative importance of which must be determined before generalizations are made.

The differences shown between the geographically separated lines of the same variety when brought together in the locality where one of the lines has been grown may be ascribed to three general causes:

(1) Cross-pollination. Varieties removed to different localities may become crossed with other sorts, with the result that their characteristics are changed in a greater or less degree.

(2) Different standards of selections. Changed conditions in connection with the diversity that exists in all varieties bring slightly different types of plants into prominence, and selection, either conscious or unconscious, results in a changed type.

(3) New-place effects. The more or less temporary changes that follow a transfer to new conditions, caused by the novelty or "shock" of the new environment without special reference to the nature of the change.

So far as known, new-place effect, which the experiments here reported show to be a significant factor in the transfer of seed, has not previously been considered with regard to maize.

These experiments were originally planned with the idea that the seed of a first-generation hybrid might be transferred to a distant locality without showing the reduced yields thought to follow the transfer of pure strains.

It was believed that with first-generation hybrid seed it might be a matter of indifference where the hybrid was made, provided the parents were suited to the localities where the crop was to be grown.[1] If found to be the case, this would constitute an important addition to the advantages that follow the use of first-generation hybrid seed, making possible a wider application of the results secured through the work of skilled breeders.

The results indicate that hybrids made in one locality and grown in another are not only at no disadvantage compared with the same hybrids produced at the locality where the comparison is made but that the introduced hybrid may even be superior. It further appears that the same is true of pure strains and that, after the effects of cross-pollination and selection have been eliminated from the problem, there is a residual effect of the transfer to the new place that tends to increase rather than to reduce the vigor and yield of the plants.

NATURE OF THE EXPERIMENTS

It was planned to conduct the experiments at the following places: Stockton, Kans.; Victoria, Tex.; and Lanham, Md. These places represent a wide range of soil and climate. At Stockton, which is in the eastern part of the semiarid Great Plains area, there is a fertile friable soil, low rainfall, low atmospheric humidity, and a prevalence of high winds. Victoria, located in the Gulf region of Texas, has a stiff black clay soil of good fertility. The total rainfall is usually large and the humidity high, though severe drouths are not uncommon. At Lanham, a few miles north of Washington, D. C., the soil is sandy, acid, and relatively infertile. The rainfall and humidity are usually neither deficient nor excessive. In the discussion of results these localities will be referred to as Kansas, Texas, and Maryland.

Four varieties of maize were chosen for the experiment, as follows:

(1) Stockton, a white dent variety developed at Stockton, Kans.

(2) Strawberry, a large-eared Texas variety with red and white variegated dent seed, well adapted to the conditions at Victoria, Tex.

(3) Hickory King, a strain of this variety grown in Virginia and well suited to the conditions at Lanham, Md.

(4) Boone, a strain of "Boone County White," the seed of which was obtained from Illinois.

In the spring of 1912 seed of the four varieties were planted at each of the three places named. The precaution was taken to mix the seed

[1] COLLINS, G. N. THE VALUE OF FIRST-GENERATION HYBRIDS IN CORN. U. S. Dept. Agr. Bur. Plant Indus. Bul. 191, p. 33. 1910.

of each of the kinds so that the portions sent to the several localities should be as nearly alike as possible. The order of planting was the same at all the places, as follows: Every alternate row was planted to the Boone variety, which was used as the male parent in making hybrids. The seed of each of the four varieties, including the Boone itself, was planted in the rows alternating with the Boone.

The Boone plants standing in the alternate rows throughout the field were allowed to shed pollen. All others, including the interplanted Boone, were detasseled, so that the only pollen shed was from the Boone variety. Seed was saved from the detasseled rows only. This was of four kinds. (1) Stockton × Boone, (2) Strawberry × Boone, (3) Hickory King × Boone, and (4) cross-pollinated seed of the Boone. At each locality the experiment was placed at a distance from all other corn.

In 1913 it was planned to compare the behavior of the plants raised from the seed produced at the three localities when grown at each of these places. Although grown in the same field, no attempt was made to compare the relative merits of the several hybrids, each hybrid together with the pure-seed Boone constituting a separate experiment involving only the comparison of the yield from the seed of the three localities. Thus, there were four experiments to be made at each of the three places. Since the arrangement was the same at all places, one description will suffice.

To compare the Stockton × Boone hybrid from the three localities, the seed from the several places were planted in adjoining rows, the first row from the Kansas seed, the second from the Texas seed, and the third from the Maryland seed. The series was repeated 10 times, making 10 distinct comparisons. A similar procedure was followed with the three other hybrids and with the pure-seed Boone.

At Stockton, Kans., excessive drouth destroyed the entire corn crop. Since no results were secured from Stockton, the behavior of the Kansas-grown seed will be eliminated from the discussion of the results, which will be confined, therefore, to the experiments conducted in Texas and Maryland.

At Victoria, Tex., the rows were 100 feet long. The seed was drilled, and the plants were thinned to about 2 feet in the row. When harvested, a weighed sample of 20 pounds of ears was saved from each row. This sample was thoroughly air-dried, after which it was again weighed to determine the loss of water. The percentage of grain to cob was also determined. No significant differences in water content or percentage of grain were found in the crops from the seed from different localities, and these determinations are, therefore, not discussed.

At Lanham, Md., the seed was planted in hills 3 feet apart, in rows 132 feet long. The plants were thinned to one stalk per hill. The method of harvesting was similar to that of Victoria except that no determinations of dry weight were made.

At each locality the corn from all the experiments was harvested the same day; and the weight of ears, together with the number of plants, was recorded for each row.

To avoid, so far as possible, differences due to inequalities of soil and to obtain reliable averages, each pair of rows consisting of one row each of Maryland- and Texas-grown seed was considered a separate test. The relative behavior of the plants from the two sources of seed was determined by an average of all the comparisons, usually 10 in number.

In Table I are given the yield in pounds per row and the yield per plant from the several rows. Yields which stand opposite in the table are from adjoining rows in the field.

TABLE I.—*Behavior of plants from Maryland- and Texas-grown seed subsequently planted in Maryland and Texas*

STOCKTON × BOONE

Variety and factor.	Compared at Lanham, Md.			Compared at Victoria, Tex.		
	Yield from seed produced at Lanham.	Yield from seed produced at Victoria.	Yield from seed produced at Lanham expressed as a percentage of the mean.	Yield from seed produced at Lanham.	Yield from seed produced at Victoria.	Yield from seed produced at Lanham expressed as a percentage of the mean.
	Pounds.	*Pounds.*	*Per cent.*	*Pounds.*	*Pounds.*	*Per cent.*
Yield per plant	0.90	1.15	87.8	0.65	0.65	100.0
Do	.90	1.06	91.8	.65	.66	99.2
Do	.92	.93	99.4	.62	.56	105.1
Do	.93	.81	106.9	.63	.81	87.5
Do	.89	.74	109.2	.65	.63	101.5
Do	.60	.52	107.1	.62	.69	94.6
Do	.26	.61	59.8	.64	.66	98.5
Do	.58	.56	101.7	.59	.62	97.5
Do	.78	.78	100.0	.64	.64	100.0
Do	.67	.71	97.1	.64	.66	98.5
Average	.743	.787	96.08±2.75	.633	.658	98.25±0.86
Yield per row	30.5	31.0	99.1	32.5	33.0	99.2
Do	32.5	41.5	87.8	31.8	32.5	98.9
Do	33.0	32.5	100.8	32.0	32.0	100.0
Do	25.0	29.0	92.6	32.8	38.0	92.6
Do	29.5	18.5	122.9	34.0	31.5	103.8
Do	9.0	11.0	90.0	28.5	36.5	87.7
Do	5.5	19.5	44.0	34.5	30.5	106.2
Do	18.0	18.5	98.6	31.0	36.5	91.9
Do	23.5	26.5	94.0	34.8	34.5	100.4
Do	22.0	22.0	100.0	38.0	33.0	107.0
Average	22.85	25.0	92.99±3.25	32.97	33.80	98.76±1.64

STRAWBERRY × BOONE

Yield per plant	1.80	1.92	96.8	0.70	0.51	115.7
Do	1.81	1.61	105.9	.72	.72	100.0
Do	1.92	1.74	104.9	.78	.84	96.3
Do	1.89	1.64	107.1	.80	.84	97.6

TABLE I.—*Behavior of plants from Maryland- and Texas-grown seed subsequently planted in Maryland and Texas*—Continued

STRAWBERRY × BOONE—continued

Variety and factor.	Compared at Lanham, Md.			Compared at Victoria, Tex.		
	Yield from seed produced at Lanham.	Yield from seed produced at Victoria.	Yield from seed produced at Lanham expressed as a percentage of the mean.	Yield from seed produced at Lanham.	Yield from seed produced at Victoria.	Yield from seed produced at Lanham expressed as a percentage of the mean.
	Pounds.	*Pounds.*	*Per cent.*	*Pounds.*	*Pounds.*	*Per cent.*
Yield per plant....	1. 83	1. 65	105. 2	0. 74	0. 82	94. 9
Do...........	1. 72	1. 38	111. 0	. 74	. 85	93. 1
Do...........	....	....	....	. 77	. 74	102. 0
Do...........	....	....	....	. 69	. 78	93. 9
Do...........	....	....	....	. 71	. 65	104. 4
Do...........	....	....	....	. 70	. 72	98. 6
Average....	1. 83	1. 66	105. 15±1. 09	. 735	. 747	99. 65±1. 32
Yield per row......	75. 5	71. 0	103. 07	39. 00	27. 30	117. 7
Do...........	63. 5	64. 5	99. 22	42. 50	30. 30	116. 8
Do...........	71. 0	61. 0	107. 58	41. 50	32. 00	112. 9
Do...........	68. 0	62. 5	104. 21	39. 00	34. 50	106. 1
Do...........	62. 0	61. 0	100. 81	37. 00	37. 50	99. 3
Do...........	67. 0	47. 0	117. 54	36. 80	37. 50	99. 0
Do...........	....	....	....	39. 30	33. 30	108. 3
Do...........	....	....	....	37. 00	39. 80	96. 4
Do...........	....	....	....	37. 00	30. 50	109. 6
Do...........	....	....	....	38. 00	38. 00	100. 0
Average....	67. 8	61. 2	105. 40±1. 80	38. 70	34. 05	106. 61±1. 82

HICKORY KING × BOONE

Variety and factor.	Lanham: Lanham seed	Lanham: Victoria seed	Lanham: per cent	Victoria: Lanham seed	Victoria: Victoria seed	Victoria: per cent
Yield per plant....	0. 63	0. 58	104. 1	0. 71	0. 57	110. 9
Do...........	. 51	. 60	91. 9	. 60	. 58	101. 7
Do...........	. 53	. 60	93. 8	. 57	. 57	100. 0
Do...........	. 86	. 97	94. 0	. 62	. 57	103. 9
Do...........	1. 18	1. 15	101. 3	. 58	. 53	104. 5
Do...........	1. 25	1. 08	107. 3	. 62	. 57	104. 2
Do...........	1. 29	1. 50	92. 5	. 56	. 43	113. 1
Do...........	1. 37	1. 24	105. 0	. 56	. 57	99. 1
Do...........	1. 20	1. 36	93. 8	. 63	. 51	110. 5
Do...........	1. 34	1. 30	101. 5	. 61	. 55	105. 2
Average....	1. 016	1. 038	98. 22±1. 42	. 606	. 545	105. 31±1. 0
Yield per row......	21. 5	21. 0	101. 2	31. 00	21. 00	119. 2
Do...........	17. 5	18. 5	97. 2	29. 00	23. 00	111. 5
Do...........	17. 5	18. 0	98. 6	28. 00	25. 00	105. 7
Do...........	32. 0	40. 5	88. 3	32. 30	27. 30	108. 4
Do...........	46. 0	38. 0	109. 5	31. 30	27. 50	106. 4
Do...........	50. 0	40. 0	111. 1	28. 00	26. 00	103. 7
Do...........	51. 5	58. 5	93. 6	28. 80	23. 00	111. 1
Do...........	52. 0	46. 0	106. 1	32. 80	24. 00	115. 4
Do...........	47. 0	49. 0	97. 9	31. 50	25. 50	110. 5
Do...........	51. 0	52. 0	99. 0	28. 50	26. 50	103. 6
Average....	38. 6	38. 15	100. 25±1. 43	30. 10	24. 87	109. 55±1. 13

TABLE I.—*Behavior of plants from Maryland- and Texas-grown seed subsequently planted in Maryland and Texas*—Continued

BOONE

Variety and factor.	Compared at Lanham, Md.			Compared at Victoria, Tex.		
	Yield from seed produced at Lanham.	Yield from seed produced at Victoria.	Yield from seed produced at Lanham expressed as a percentage of the mean.	Yield from seed produced at Lanham.	Yield from seed produced at Victoria.	Yield from seed produced at Lanham expressed as a percentage of the mean.
	Pounds.	*Pounds.*	*Per cent.*	*Pounds.*	*Pounds.*	*Per cent.*
Yield per plant	1.38	1.36	100.7	0.56	0.78	83.6
Do	1.15	1.21	97.5	.60	.73	90.2
Do	1.08	1.36	88.5	.58	.68	92.1
Do	1.30	1.26	101.6	.63	.67	96.9
Do	1.10	1.13	98.7	.60	.64	96.8
Do	1.35	1.11	109.8	.69	.62	105.3
Do	1.22	1.51	89.4	.60	.68	93.8
Do	1.30	1.35	98.1	.62	.63	99.2
Do	1.16	1.22	97.5			
Do	1.14	1.24	95.8			
Average	1.218	1.275	97.76±0.91	.610	.678	94.74±1.54
Yield per row	41.5	45.0	95.95	30.50	27.00	106.1
Do	42.5	46.0	96.05	29.50	32.30	95.6
Do	45.5	47.5	97.85	33.50	32.00	102.3
Do	53.5	48.0	105.42	34.00	30.00	106.3
Do	39.5	43.0	95.76	34.50	30.00	107.0
Do	44.5	41.0	104.09	32.00	29.30	104.7
Do	47.5	53.0	94.53	32.50	28.00	107.4
Do	48.0	46.0	102.13	31.25	32.00	98.8
Do	44.0	47.5	96.17			
Do	41.0	49.5	90.61			
Average	44.75	46.65	97.87±1.02	32.22	30.06	103.5±1.50

The yield of the plants from the Maryland- and Texas-grown seed is made comparable by expressing the yield of the former as a percentage of the mean yield of both. For example, in Table I where the behavior of the Stockton × Boone hybrid is considered, the yield per plant of the first row at Lanham, Md., which was from Maryland-grown seed, is shown as 0.90 pound; the adjoining row from Texas-grown seed yielded 1.15 pounds per plant. The mean of the yield of these two rows is 102.5 pounds, and the yield from the Maryland-grown seed is 87.8 per cent of this mean, the value given in the fourth column. The average of the 10 comparisons is, in this case, 96.08 per cent—that is, the yield of the plants from the Maryland-grown seed averages 3.92 per cent below the mean yield of this strain grown in Maryland. This expression for the relative behavior in Maryland of the plants from the Maryland-grown seed is to be compared with the results of the similar comparison made in Texas, given in the next three columns. From these it is seen that in Texas the yield per plant of the plants from Maryland-grown seed was 98.25 per cent of the mean yield of the strain.

At both localties the Maryland-grown seed of this cross is inferior to that produced in Texas, but the point to which attention is now directed is that the inferiority is greater in Maryland than in Texas.

Table II contrasts the average behavior of each of the three hybrids and the pure-seed Boone at the two localities. For example, when compared in Maryland, the yield per row of plants from the Maryland-grown seed of Stockton × Boone averaged 92.99 per cent of the mean yield of the cross. In Texas the same comparison showed the average yield of plants from Maryland-grown seed to be 98.76 per cent of the mean. Thus, the plants from Maryland-grown seed averaged 5.77 per cent higher in yield in Texas than in Maryland.

TABLE II.—*Average behavior of Maryland-grown seed expressed as a percentage of the mean of Maryland- and Texas-grown seed, 1913*

Factor and kind of seed.	Compared in Maryland.	Compared in Texas.	Comparative increase of Maryland-grown seed in Texas.	Increase divided by probable error.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
Yield per plant:				
Stockton × Boone	96. 08±2. 75	98. 25±0. 86	2. 17±2. 88	0. 75
Strawberry × Boone	105. 15±1. 09	99. 65±1. 32	−5. 50±1. 71	−3. 22
Hickory King × Boone	98. 22±1. 42	105. 31±1. 05	7. 09±1. 76	4. 03
Boone	97. 76±0. 91	94. 74±1. 54	−3. 02−1. 69	−1. 69
Yield per row:				
Stockton × Boone	92. 99±3. 25	98. 76±1. 64	5. 77±3. 64	1. 59
Strawberry × Boone	105. 40±1. 80	106. 61±1. 82	1. 21±2. 56	. 47
Hickory King × Boone	100. 25±1. 43	109. 55±1. 13	10. 01±1. 58	6. 34
Boone	97. 87±1. 02	103. 50±1. 50	5. 63±1. 81	3. 11

In comparing the yields both the yield per row and the yield per plant were considered. In Texas the yield per row was much more dependent on the number of plants in the row than in Maryland. In Texas, as the number of plants increased, there was a pronounced tendency for the yield per row to increase and the yield per plant to decrease, while in Maryland an increase in the number of plants in the row was accompanied by only a small increase in the yield per row, and there was an almost corresponding increase in the yield per plant.

This difference in behavior at the two localties is not difficult to understand. In Maryland the failure to secure a perfect stand was largely the result of infertile spots in the field, and the same unfavorable conditions which reduced the number of plants also reduced the yield of those that survived. In Texas, the loss of plants was more the result of accidental factors, which influenced the yield of the remaining plants only by permitting them to take advantage of the increased space with a consequent slight increase in the yield per plant. The method of planting in Texas accentuated this difference, for with the plants close together in the row the additional space resulting from a missing plant was utilized more by the neighboring plants in the same row than by those in adjoining rows. In Maryland, on the other hand, where the plants were spaced equally in both directions, half the space made available by a missing hill would be appropriated by the adjoining rows.

TABLE III.—*Stand of plants secured from Maryland and Texas grown seed compared in Maryland and Texas*

Variety.	Compared in Maryland.			Compared in Texas.		
	Number of plants from Maryland-grown seed.	Number of plants from Texas-grown seed.	Stand of Maryland-grown seed expressed as percentage of mean.	Number of plants from Maryland-grown seed.	Number of plants from Texas-grown seed.	Stand of Maryland-grown seed expressed as percentage of mean.
Stockton × Boone	34	27	111.5	50	51	99.0
Do	36	39	96.0	49	49	100.0
Do	36	35	101.4	52	57	95.4
Do	27	36	85.7	52	47	105.1
Do	33	25	113.8	52	50	102.0
Do	15	21	83.3	46	53	92.9
Do	21	32	79.2	54	46	108.0
Do	31	33	96.9	53	59	94.6
Do	30	34	93.8	54	54	100.0
Do	33	31	103.1	59	50	108.3
Average	......	......	96.5±2.50	......	......	100.5±1.20
Strawberry × Boone	42	37	106.3	56	53	102.8
Do	35	40	93.3	59	42	116.8
Do	37	35	102.8	53	38	116.5
Do	36	38	97.3	49	41	108.8
Do	34	37	95.8	50	46	104.2
Do	39	34	106.8	50	44	106.4
Do	......	......	......	51	45	106.3
Do	......	......	......	54	51	102.9
Do	......	......	......	52	47	105.1
Do	......	......	......	54	53	100.9
Average	......	......	100.4±1.86	......	......	107.1±1.18
Hickory King × Boone	34	36	97.1	44	37	108.6
Do	34	31	104.6	48	40	109.1
Do	33	30	104.8	49	44	105.4
Do	37	42	93.7	52	48	104.0
Do	39	33	108.3	54	52	101.9
Do	40	37	103.9	45	46	98.9
Do	40	39	101.3	51	54	97.1
Do	38	37	101.3	58	42	116.0
Do	39	36	104.0	50	50	100.0
Do	38	40	97.4	47	48	98.9
Average	......	......	101.6±.98	......	......	104.0±1.31
Boone	30	33	95.2	54	36	120.0
Do	37	38	98.7	49	44	105.4
Do	42	35	109.1	58	47	110.5
Do	41	38	103.8	54	45	109.1
Do	36	38	97.3	57	47	109.6
Do	33	37	94.3	46	47	98.9
Do	39	35	105.4	54	41	113.7
Do	37	34	104.2	50	51	99.0
Do	38	39	98.7	......	......	......
Do	36	40	94.7	......	......	......
Average	......	......	100.1±1.24	......	......	108.3±1.72

Ability to produce a stand may legitimately be considered one of the manifestations of greater vigor. That it is a definite and positive factor is shown in Tables III and IV, in which the relative stands are compared. With all four kinds a comparison of the relative stand at the two localities is in favor of the transferred seed. In the Boone variety the transfer of seed has resulted in an 8 per cent increase of stand, a difference nearly four times the probable error. Since the analysis of the comparative stand of local and transferred seed shows that the differences are not accidental, but are consistently in favor of the transferred seed, it would seem that yield per row is a more reliable measure of comparative vigor than yield per plant. Yield per row is the measure of the practical results, and from this standpoint it is seen that all four strains showed an increase in yield as a result of transfer of seed. In Texas, where there was a definite tendency for an increased number of plants in a row to reduce the yield per plant, yield per plant is obviously ill calculated to bring out the real difference in vigor.

TABLE IV.—*Average stand of plants secured from Maryland-grown seed expressed as a percentage of the mean of Maryland and Texas-grown seed*

Kind of seed.	Compared in Maryland.	Compared in Texas.	Increased stand of Maryland-grown seed in Texas.	Increase divided by probable error.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
Stockton × Boone	96. 5±2. 50	100. 5±1. 20	4. 0±2. 77	1. 44
Strawberry × Boone	100. 4±1. 86	107. 1±1. 18	6. 7±2. 20	3. 04
Hickory King × Boone	101. 6±0. 98	104. 0±1. 31	2. 4±1. 63	1. 47
Boone	100. 1±1. 24	108. 3±1. 72	8. 2±2. 12	3. 87

EXPERIMENTS IN 1915 AND 1916

The results of the 1912 and 1913 experiments were so at variance with current belief that it was thought best to obtain additional evidence before publishing. A somewhat similar experiment was therefore planned and carried out during the years 1915 and 1916. The same varieties were used as in the previous experiment, but the localities were changed by substituting Greenville, Tex., and Sacaton, Ariz., for Victoria, Tex., and Stockton, Kans.

Crop failure at Greenville again limited the experiment to two localities: Lanham, Md., and Sacaton, Ariz. At Sacaton the temperatures are high, and there is practically no rainfall during the growing season, the crop being grown by means of irrigation.

To eliminate differences due to irregularities in the stand of plants, a different system of planting was adopted. Seed from both localities were planted in each hill, the seed from the two sources being identified by their positions in the hill. At harvest the measurements were confined to the hills which contained plants from both Maryland- and Ari-

zona-grown seed. For all such hills the height of each plant was recorded with the total length of the ear or ears. In this way each hill constituted a unit of comparison. The height of each plant was expressed as a percentage of the mean height of the two plants in the same hill. These determinations were then averaged to secure an expression of the mean behavior of the plants from each source of seed. Length of ear was treated in the same way. Table V gives the results. Unfavorable conditions so reduced the yields at Lanham, Md., that length of ear was recorded for only three of the strains, and even for these there was so much variation that the results are of doubtful significance. They serve, however, to supplement the results on the height, with which they are in accord.

TABLE V.—*Average behavior of Maryland-grown seed expressed as a percentage of the mean of Maryland- and Arizona-grown seed, 1916*

Factor.	Kind of seed.		Compared in Maryland.	Compared in Arizona.	Increase of Maryland-grown seed in Arizona.	Increase divided by probable error.
	Produced in Maryland, 1915.	Produced in Arizona, 1915.				
			Per cent.	*Per cent.*	*Per cent.*	*Per ct.*
Height of plants.	Stockton×Boone	Stockton×Boone	92.9±1.21	98.9±0.38	6.0±1.27	4.72
Do	Boone×Stockton	do	92.7±0.85	99.7±0.43	7.0±0.95	7.37
Do	Strawberry×Boone	Strawberry×Boone	89.2±1.74	97.1±0.58	7.9±1.83	4.32
Do	Boone×Strawberry	do	95.7±1.44	100.9±0.46	5.2±1.50	3.47
Do	Hickory King × Boone.	Hickory King × Boone.	103.5±0.53	97.5±0.46	−6.0±0.70	−8.57
Do	Boone×Self	Boone×Boone	108.1±0.90	109.0±0.56	0.9±1.06	0.85
Length of ear.	Boone×Stockton	Stockton×Boone	88.8±7.6	103.9±2.7	15.1±8.1	1.9
Do	Hickory King × Boone.	Hickory King × Boone.	112.4±2.2	105.1±3.3	−7.3±4.0	1.8
Do	Boone×Self	Boone×Boone	146.3±6.0	159.4±2.9	13.1±5.7	2.3

At Sacaton, Ariz., in 1915 reciprocal crosses were made with Stockton×Boone and Strawberry×Boone, and these reciprocals were separately compared with the seed grown at Lanham, where Boone was used only as the male parent. In all of the six comparisons except one, transferring the seed resulted in increased height; and in all but one the difference is almost certainly not the result of chance.

In 1915 the crosses in both Maryland and Arizona were made by hand instead of by detasseling alternate rows as in 1912. In gathering pollen an effort was made to obtain pollen from as many plants as possible and, so nearly as might be, in equal amounts from each plant. In like manner selection of female parents was avoided so far as possible. In spite of these precautions, it is evident that there would still be a measure of selection. Some plants produce virtually no pollen, and many plants fail to develop an ear. Furthermore, since the plants were thinned to a stand of one in a hill from each locality, obviously weak plants being removed, it would seem that here too there would be a tendency to retain

the types of plants best adapted to the conditions where the experiment was tried. The entire effect of selection would be to favor the home-grown seed, and that the transferred seed was not superior to the home-grown in every instance may not be held to vitiate the cases in which significant differences in favor of the transferred seed were observed.

The results indicate, however, that the stimulation is more pronounced in some stocks than in others. Thus, in the 1916 comparisons Boone × Hickory King stands out as a conspicuous exception. In all other stocks the transferred seed produced taller plants than the home-grown seed; but with Boone × Hickory King, the home-grown seed exceeded the transferred by 6.2 per cent, a difference not to be ascribed to chance, being more than eight times the probable error. Of the three stocks in which the yield was taken, Boone × Hickory King is also the only one to show superiority for the home-grown seed. Taken alone, the differences in yield could not be considered significant, but the agreement with the results for height confirms the reliability of these results.

The insignificant increase in the case of Boone may be explained by the fact that the Arizona-grown seed was more closely selected to fit the Arizona conditions than were the other kinds. At Sacaton the pure-seed Boone was obtained by selfing. This procedure would restrict the plants from which seed was secured to those able to produce both ears and pollen under Arizona conditions. With cross-pollinated seed and hybrids, plants that produced no ears would be represented in the progeny as male parents.

DISCUSSION OF RESULTS

Three classes or degrees of new-place effects ("neotopism") have been recognized by Cook [1], chiefly with reference to cotton: (1) Those in which there is merely a stimulation of growth; (2) those in which there is also a definite general change of the hereditary characteristics of the variety; and (3) those in which the new conditions call forth a promiscuous mutative diversity.

The results here reported give evidence under the first of these categories only. With respect to the more pronounced changes that follow the transfer of varieties from the Tropics to a temperate climate it may be said that many such changes do occur in maize, some of which at least are inherited. For conclusive qualitative evidence on this point, however, there is lacking definite information regarding the behavior of the introduced varieties in their native countries.

Roberts [2] has pointed out that the striking effects which have been ascribed to acclimatization in maize are to be referred either to cross-pollination with native varieties or to the results of selection. The effect of cross-pollination, which misled early investigators, has presumably

[1] Cook, O. F. Aspects of kinetic evolution. *In* Proc. Wash. Acad. Sci., v. 8, 1906, p. 236. 1907.

[2] Roberts, H. F. Acclimatization with reference to corn breeding. *In* 1st Ann. Rpt. Kans. Corn Breeders' Assoc., [1905]/06, p. 60–64. 1906.

been eliminated from recent experiments, but the effects of selection are so pronounced and speedy that in experiments hitherto reported any direct effect of the environment on the characters of the plants would be completely masked. The characteristics of a maize variety are altered readily by selection. When grown in a new locality for a few years, even without conscious selection, the type may change rapidly; and when brought back to the original locality, it is in reality a different variety. The characters brought into prominence in the new locality may render the stock less suited to the old conditions, though better adapted to the new.

It would be very difficult, if not impossible, to eliminate completely all selective action. Even when all seed is saved, those individuals or types of plants which are best adapted to the conditions under which they are grown will produce a greater proportion of the seed than will the types which are less well adapted, and those least adapted may produce no seed at all. In two localities where different conditions prevail, the highest yielding plants—hence those contributing the largest proportion of the seed—would presumably be of different types; and when brought together and compared, we should expect to find a slight advantage for the locally grown seed. Yet the results of the present experiments indicate that the effect of selection during a single season may be so slight as not to mask completely the opposing new-place effect.

Since new-place effect in maize seems to operate as a stimulus, it would tend to obscure any lack of adaptation in newly introduced varieties. The recognition of new-place effect may be said, therefore, to increase rather than diminish the importance that must be assigned to adaptation.

As a result of the stimulation due to new-place effect, the cultivation of an inferior strain might be extended as a result of its satisfactory performance the first year following its introduction.

The stimulus that followed the transfer of seed in these experiments is doubtless similar to the increased vigor imparted to many vegetables by growing the crop in localities remote from the place where the seed was produced. The economic utilization of increased vigor secured in this way is usually confined to crops which are grown for the sake of some part other than the seed. In cotton, for example, the increase of vigor in the plants following a transfer of seed is often very pronounced, although the crop of seed and fiber may be reduced. In maize, as a result of the determinate habit of the plant, vegetative vigor and seed production are more closely associated, so that the possibility of practical utilization seems greater.

CONCLUSIONS

Hybrids between the same pairs of varieties made at different localities showed no decrease in yield as a result of transferring the first-generation seed to a new locality. On the contrary, the change of

environment seemed to act as a stimulus, with the result that the yields were increased in all but one of the hybrids tested. One unhybridized variety was included in the experiment, and this also gave slightly increased yields as a result of being transferred to a new environment.

In 6 of the 10 comparisons the increase is too large to be ascribed to experimental error and indicates that new-place effect should be taken into consideration as a factor of production.

That significant increases may be secured by taking advantage of new-place effect in maize should not be used as an argument in favor of the general transfer of seed. There is no evidence that the importance of using acclimatized seed has been overestimated. On the contrary, the experiments show that new-place effect may often obscure the differences between acclimatized and unacclimatized seed when first compared, and thus interfere with a full appreciation of the value of adaptation.

The investigations show the existence of a hitherto-neglected factor in maize production, but much more extensive experiments are needed to ascertain the extent and practical importance of this factor. The existence of one definite exception indicates that the tendency to increased vigor following a transfer of seed is not universal. The results also indicate that adaptation in maize comes about through selection rather than as a direct reaction to the environmental conditions.

RELATION OF THE VARIABILITY OF YIELDS OF FRUIT TREES TO THE ACCURACY OF FIELD TRIALS[1]

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INTRODUCTION

The value of the outcome of any trial depends upon the probability that a similar result will be obtained if the trial is repeated. In recent years the agricultural experiment stations of all countries have greatly increased the number and size of their field trials. A casual examination of such trials usually shows a wide range in the reliability of the results. The yields of control plots in different parts of the same tract will often differ as much among themselves as the yields of fertilized and unfertilized plots differ from each other. The purpose of this paper is to present the results of a study of the variation in recorded yields of fruit trees taken singly and in groups of various sizes, and especially to determine the effect upon variability of various combinations and repetitions of unit plots.

It is recognized that the results of a single experiment are often untrustworthy; yet experimenters have published single results and have based practical advice upon them. However well planned field trials may be, the interpretations of the results can hardly be considered of infinite reliability—that is, results which will invariably be obtained when the trials are repeated. The best that can be done is to construct the most probable results from the more or less varying observed results of individual trials. If it is impossible to obtain perfect accuracy, it is not impossible to fix the limits of error and thus to determine whether the differences obtained are due to the treatments applied or to unavoidable errors—that is, whether the differences are significant. A number of trials are necessary before a reasonably reliable result can be obtained. The average result of a series does not always represent the truth. When averages are used, they should always be accompanied by their probable errors, which are a measure of their reliability.

Before attempting to interpret the results of a plot experiment with fertilizers, it is necessary to know whether the differences observed are any greater than those which might have occurred had none of the plots been fertilized. The scientific method is to recognize the inevitable error and, while reducing it by every possible precaution, at the same time to

[1] Paper 44, University of California, Citrus Experiment Station, Riverside, Cal.

[2] The writers wish to make acknowledgment of their indebtedness for aid and criticism to Prof. H. H. Love, of Cornell University; Prof. E. B. Babcock, Dr. H. B. Frost, and other members of the University of California.

measure its probable amount so as to make sure it is not likely to vitiate our conclusions.

One of the chief difficulties in obtaining reliable results in field trials is the natural variability of the material with which we are dealing. Crops are living organisms with inherent tendencies to vary, even though it were possible to make their environmental conditions identical.

In agronomic experiments the number of plants taken is usually so large that inherent variability ceases to be a factor of importance. In horticultural experiments, however, where fruit trees are under observation, the limited number of trees possible to include in a plot may make the factor of inherent variability an important consideration.

Further variation is induced as a result of the many factors of the environment which are beyond the control and possibly the recognition of the experimenter. Some of these factors are independent; others react upon one another. In designing a set of field trials, we try to avoid, as far as possible, all secondary factors which may exert a disturbing influence.

Lack of uniformity in both the physical and chemical characteristics of the soil is one of the foremost factors causing variation in productivity of plants. Apparently uniform surface soils may be underlain with a heterogeneous subsoil. Differences also occur which are not evident on a careful inspection of both the soil and the crops, but which are easily measured by weighing the yields. In other words, the weighing machine is more sensitive than the eye and reveals differences that mere inspection can not detect.

The past treatment of the soil brings in variables the significance of which may not be comprehended at the time a field trial is begun. The persistent effects left by the application of stable manure on some of the Rothamsted plots show how large a part is played by the past history of the field. Plots which for 40 years have had identical treatment still give different crop yields because of the effect of dressings of barnyard manure applied at an earlier period.

Unequal prevalence of diseases and insects may bring about further error in the results.

Besides the above sources of variation and possibly outweighing them at times is the effect of season. No season is entirely normal; and it is only when the experiment has been repeated for several years, or, in other words, until "a fair sample of seasons" has been made, that any sort of allowance can be made for seasonal effects.

As is shown by observations at Rothamsted, it is not possible to establish a schedule of relative yields for a series of plots, even after several years' comparison. In this case two grass plots were treated alike for 50 years; by taking the yield of one plot as the standard, the yield on the other in the same season has been as low as 90 per cent and as high as 196 per cent.

Recent investigations, which are reviewed in succeeding pages, have thrown light upon the nature of the variability inherent in experiments conducted with groups of plants or of animals. So far as the writers know, however, few studies have yet been published upon the uncertainty with which one deals in attempting to carry on plot trials with orchard trees. In connection with plans for extending the trial plots of this Experiment Station, the writers have attempted to study the question of variability of tree yields and to formulate some plan to determine the probable reliability of the results. It is highly desirable to make the tree plots as small as possible without sacrificing too much accuracy on account of the large amount of land required for each individual.

A fruit tree is possibly more affected by environmental conditions than an annual crop growing from seed to maturity in one season. The tree roots penetrate more deeply into the soil and may be affected by soil differences to a considerable depth. Climatic conditions during the resting period may have marked influence on the crop production of the trees.

Pruning also introduces variation. Uniform pruning is desirable; yet different trees need different types of pruning, and more uniform results will be obtained if this is recognized.

Individual trees have apparent idiosyncrasies in fruiting habits. A tree may yield large crops of fruit in alternate years, with very light crops in intervening years. The character of the stock upon which the particular variety was budded to form the tree may profoundly influence the type, habit, and productivity of the adult tree. Furthermore, we can not overlook the possibility of errors in yields due to predacious animals which devour or otherwise destroy more or less fruit.

Fruit trees therefore present opportunity for more variability than would be expected in the growth of annual plants.

PREVIOUS STUDIES

The varying productivity of fruit trees has been called to our attention by the writings of Macoun (*1904*),[1] Munson (*1907*), Shamel (*1912*), Hedrick (*1912*), Stewart (*1913, p. 552–554*), Fletcher (*1913*), Coit (*1910*), Whitten (*1915*), Lewis and Vickers (*1915, p. 30–31*), Barre (*1915*), Gourley (*1915, p. 72–73*), Kraus (*1916*), and others. The above observations have been largely made in connection with plant breeding and orchard management problems, and were not made to bear necessarily on the accuracy of plot trials.

Holtsmark and Larsen (*1906*) were among the first to call attention to the errors of field trials. They recognized the inevitable variation of field results, and showed how it may be estimated by the use of the standard deviation and the coefficient of variability. They also showed

[1] Bibliographic citations in parentheses refer to "Literature cited", pp. 282–283.

that the coefficient of variability decreases as the plots are enlarged, but not proportionally to the size of the plot.

The limitations of field experiments were discussed by Carleton (*1909*), who called the attention of experimenters to the various uses of control plots, and to the general precautions necessary to obtain reliable results.

Hall (*1909*), Mercer and Hall (*1911*), and Hall and Russell (*1911*) recorded extensive studies of the soil variations in experimental grounds and the influence of size and repetition of plots upon accuracy. This work was done largely with the yields of wheat, mangel, and hay crops. The conclusions from the above work are that the error in field trials diminishes as the size of the plot increases, but that the reduction is small when the plot is enlarged to a size greater than one-fortieth of an acre. The error may be further diminished by increasing the number of plots similarly treated and scattering them about the area under experiment; but there is not much to be gained by increasing the number of plots above five.

Wood and Stratton (*1910*) sounded notes of caution concerning the interpretation of experimental results. Frequency distribution is discussed from the point of view of its bearing on the reliability of averaging results. The applications of the probable-error methods to questions of sampling for analysis, to field experiments, and to feeding experiments are illustrated. The probable error of field experiments was investigated by two independent methods and found to be about 5 per cent of the mean yield. Tables are given showing the number of duplicate plots or number of animals in a feeding trial which must be employed to give any desired precision in the result. It is shown that more accurate results may be obtained by employing large numbers of small scattered plots than by using one large plot.

The estimation of errors in field-plot tests has been given considerable attention by Lyon (*1912*) and coworkers. It was shown that it is not possible to establish a schedule of relative yields for a series of plots, even after several years' comparison. Also, there seems to be little gain by using plots larger than one-fiftieth of an acre in size when the comparative yield of the crops is made the criterion. An area of one-twenty-fifth of an acre of land distributed in four widely separated plots, devoted to any one test, secures a much greater degree of accuracy than the same area of land in one body. The probable error was reduced from 4.5 to 2 per cent by such distribution.

Pickering (*1911*), from studies on apples and pears, concluded that experimental plots should include 6 to 12 fruit trees. Precautionary advice was also given concerning the measurement of results by crop production, foliage, and tree characteristics. In comparing the results on the treated plots with the controls, instead of taking the average of the controls, he prefers to plot these results out and to draw a smoothed

curve through them, and then to compare the results of the experimental plots with readings taken at corresponding points of this curve.

Wood (*1911*) showed how the degree of reliance can be determined for any set of experimental results by the use of the probable error. The use of this constant was demonstrated in interpreting laboratory analyses, as well as both plot and feeding experiments. Working with mangel yields, the author calculates the number and size of plots required to attain any desired precision, and working with the probabl eerror of live-weight increase of sheep, tables are given showing the number of animals required in an experiment to attain various degrees of reliability.

Several papers by Harris (*1912*, *1913a*, *1913b*, and *1915*) have drawn our attention to several phases of the experimental error in field tests. A measure of the variability of the soil productivity is obtained by determining the correlation between the yields of ultimate small plots and the yields of various groups of adjacent plots. The more nearly this correlation approaches zero, the more homogeneous the soil. This method of measurement does not seem to provide as definite a means of obtaining a corrective term as the use of the coefficient of variability and the probable error as used by Wood, Wood and Stratton, Mercer and Hall, etc., or the contingency method of correction as used by Surface and Pearl (*1916*).

Montgomery (*1912*) has also discussed the comparative variability resulting from increasing the size of the plot and from distributing small ultimate plots over the area. The latter method was found to be more accurate. In a subsequent paper (*1913*) the relative reliability of yields of wheat planted in rows and in square blocks is discussed.

An exhaustive and discriminating discussion of the nature and magnitude of variability in the results of feeding experiments has been given by Mitchell and Grindley (*1913*). Much of their discussion is equally applicable to experimentation with plants.

Olmstead (*1914*) applied the method of least squares in calculating the reliability of the yields of the mangel and wheat crop records of Mercer and Hall, the potato records of Lyon, and the wheat yields of Montgomery. The conclusions from this series of observations are:

> The estimation of the probable error of a large number of small duplicate plots well distributed in the area devoted to a field experiment indicates that the precision of agricultural experiments can be increased by replicating the experiments on small plats.

Coombs and Grantham (*1916*) have studied the variation in the yields of rice and coconuts for one year, and discussed the range and interpretation of the probable error. They showed that the yields from any two single plots could only be significant when the difference amounted to 22.8 per cent of the mean. They also introduced calculations to show the odds that any increase is a real increase and not a probable error.

The use of controls and repeated plantings in varietal tests was studied by Pritchard (*1916*) in breeding work with sugar beets. His studies lead to the conclusion that the practice of dispensing with control rows and using the mean of all progeny rows as a standard of comparison appears to be less accurate than the employment of frequent controls. However, the employment of every alternate row as a control was not sufficient to offset the variability in yield arising from irregularities of soil.

Stockberger (*1916*) discussed the value of a number of the common methods for determining the normal yield of treated plots based upon the yields of hops. Normal yields for various plots varied widely according to the method of computation, the values in some cases differing from the actual yield by as much as 40 per cent. Repetition brought about a very marked reduction in variability, although with only five repetitions the error is still relatively large.

The work of Surface and Pearl (*1916*) shows an advance in the refinement of methods of conducting field trials. With the realization that the use of frequent control plots often produces results far from satisfactory, these workers have calculated by the contingency method the probable yield of each plot of ground in their grain-testing series. This calculated yield represents the most probable yield of each plot on the supposition that they have all been planted with a hypothetical variety whose mean yield is the same as the observed mean of the field. This "calculated" yield may then be used as a basis for determining a correction factor, whereby each area must be given a handicap plus or minus the actual yield, depending upon whether the plot in question is calculated to be a low- or a high-producing area. This method of correcting the soil variation is combined with four systematically repeated plots of one-fortieth acre of each variety, and gives a high degree of accuracy.

MATERIAL USED FOR STUDY

The studies to be reported in this paper deal with the variability of fruit-tree yields. They are based upon the individual tree yields of oranges (*Citrus sinensis*), lemons (*Citrus limonia*), walnuts (*Juglans regia*), and apples (*Malus sylvestris*) from orchards which had received uniform treatments for a number of years—indeed, so far as known, from the time of planting the trees. The orchards were carefully examined, and the records for all trees which were known to be abnormal from disease or other apparent causes were eliminated. In place of the records of trees thus eliminated the average yield of the eight surrounding trees was substituted (assuming that the tree stood at the center of a square block of nine trees).

This substitution is not entirely satisfactory, yet it was felt that it was necessary in order to compute plots of homologous size and systematic arrangement. The writers found, as a matter of fact, that there is a very high degree of correlation in these orchards between the yields

of individual trees and the average of surrounding trees. For example, they found coefficients of correlation as high as 0.652 ± 0.065 for the Eureka lemons and 0.628 ± 0.060 for the Arlington navels. According to the formula given by Harris (*1915*), the correlation between individual trees and eight adjacent trees in a plot of the Arlington navels is 0.576 ± 0.04. In view of these results, the writers felt justified in using this method of substituted values.

The fruit plantations herein discussed, to judge by the surface soil, size, and condition of the trees, as well as their apparent fruitfulness, appeal to the observer as uncommonly uniform. All the orchards studied are situated in semiarid regions and are artificially irrigated during the summer months. This fact is believed to be a distinct advantage for the purpose of reducing the variability of one year's yield compared with another, since it insures a fairly uniform water supply for the soil and reduces one of the variants inevitable in nonirrigated localities.

All yields of the several fruit and nut plantations are given in pounds per tree of the ungraded product.

DESCRIPTION OF THE PLANTATIONS

NAVEL ORANGE (ARLINGTON).—These records were of the 1915–16 yields of one thousand 24-year-old navel-orange trees near Arlington station, Riverside, Cal. The individual tree production is shown by figure 1.

The grove consists of 20 rows of trees from north to south, with 50 trees in a row, planted 22 by 22 feet. A study of the records shows certain distinct high- and low-yielding areas. The northeast corner and the south end contain notably high-yielding trees. The north two-thirds of the west side contains a large number of low-yielding trees. These areas are apparently correlated with soil variation. Variations from tree to tree also occur, the cause of which is not evident. These variations, which are present in every orchard, bring uncertainty into the results of field experiments.

In making their calculations this grove was divided by the writers into imaginary plots of any size and shape desired. The yields of these plots were then compared with one another and their variability ascertained. The distribution of both the theoretical and actual yields of this grove is shown in figure 10. The yields of the individual trees when plotted according to their frequency give a skew curve of Pearson's Type I, since the critical function

$$F = \frac{\beta_1 (\beta_2 + 3)^2}{4 (4\beta_2 - 3\beta_1) (2\beta_2 - 3\beta_1 - 6)} = -0.85.$$

The distribution of the actual yields is shown on the figure by small circles. The points for the theoretical curve were calculated by the formula

$$y = y_o \left(1 + \frac{x}{l_1}\right)^{m^1} \left(1 - \frac{x}{l_2}\right)^{m^2}.$$

FIG. 1.—Diagram showing the individual tree yield (in pounds) of the navel-orange grove (Arlington).

NORTH.

Yield per tree (pounds).

Row.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Total yield per row.
1	82	77	39	114	85	60	95	94	82	71	102	108	70	108	127	64	166	156	170	83	1,953
2	74	105	78	124	81	110	131	141	122	104	117	108	116	150	131	225	199	119	180	171	2,586
3	66	69	74	79	211	89	137	23	15	107	127	163	125	140	180	168	161	203	302	277	2,716
4	75	55	84	98	132	108	147	158	101	180	83	179	105	193	148	158	106	166	255	61	2,592
5	84	71	82	109	99	127	156	110	175	159	173	196	159	39	99	152	192	268	218	142	2,810
6	48	81	51	87	69	90	136	164	112	96	46	104	136	213	197	195	191	197	184	80	2,477
7	70	81	79	109	150	90	112	121	118	158	151	116	166	131	148	180	164	182	186	156	2,668
8	54	105	82	128	101	81	135	143	198	157	211	127	148	141	115	169	180	190	238	112	2,815
9	72	82	57	86	111	75	56	153	140	107	143	180	145	161	79	75	147	156	223	172	2,420
10	28	49	48	71	84	89	33	60	165	138	126	145	149	139	107	127	152	172	32	75	1,989
11	61	31	50	65	95	75	112	112	101	120	120	131	137	217	101	123	180	124	117	171	2,243
12	68	60	100	79	98	115	110	135	74	142	90	109	175	192	182	123	173	155	35	127	2,342
13	33	80	67	69	118	64	103	190	109	210	51	78	163	116	130	101	126	121	154	69	2,152
14	36	91	107	90	80	95	78	148	107	162	161	76	123	132	114	122	152	121	167	152	2,314
15	76	109	93	89	107	49	84	164	97	124	135	147	181	183	175	90	205	167	50	146	2,471
16	49	99	81	131	109	74	123	146	156	203	146	157	156	137	180	178	140	179	159	92	2,695
17	92	123	75	45	77	61	135	93	95	224	122	178	166	170	240	208	141	168	162	90	2,665
18	34	98	89	73	123	69	139	187	164	150	163	84	111	152	176	150	150	107	130	129	2,478
19	45	57	68	37	71	83	109	45	94	139	128	141	68	79	137	90	80	130	143	107	1,851
20	53	90	128	99	72	73	80	117	137	160	152	131	116	113	169	90	57	97	110	102	2,146
21	91	80	101	117	79	135	135	62	186	132	99	80	135	134	215	105	63	85	126	69	2,229
22	73	117	83	97	93	126	116	111	115	135	105	86	103	166	164	119	115	103	152	123	2,302
23	76	83	81	84	119	67	126	96	116	111	93	44	118	165	162	34	107	21	78	28	1,809
24	148	131	86	87	98	89	107	123	108	89	57	122	102	182	135	75	65	147	86	102	2,139
25	59	66	85	71	90	58	114	90	45	116	63	99	70	117	163	22	80	71	59	59	1,597
26	86	64	78	134	174	135	111	90	93	135	112	90	152	123	149	100	33	130	114	63	2,166
27	28	76	90	144	135	111	135	116	57	190	122	128	79	165	153	164	167	195	113	89	2,457
28	51	73	79	164	161	115	127	101	71	189	180	208	140	107	154	133	107	130	103	84	2,479
29	54	82	135	141	135	90	135	160	46	120	138	90	127	82	135	63	68	91	25	5	1,922
30	162	135	155	114	163	180	209	95	163	146	164	80	110	74	54	92	90	57	103	70	2,416
31	118	147	135	120	50	95	151	61	98	148	135	189	118	130	76	82	174	88	91	107	2,313
32	103	106	62	102	135	123	135	185	148	161	180	241	151	123	95	122	135	165	137	121	2,730
33	111	69	106	158	90	104	180	145	135	117	164	180	135	161	180	135	142	160	180	90	2,742
34	135	124	135	142	140	140	239	225	193	142	156	180	218	165	213	213	180	157	152	97	3,346
35	115	100	119	164	135	169	180	196	135	112	90	159	167	90	192	139	135	135	154	69	2,755
36	127	135	116	143	135	150	214	196	169	100	180	180	164	165	225	157	195	150	251	165	3,317
37	180	104	135	148	161	135	142	164	90	154	191	135	149	116	183	173	159	210	238	107	3,074
38	118	135	135	154	141	180	160	211	161	147	198	214	200	144	215	159	77	147	214	180	3,290

39	123	159	154	181	180	150	243	293	151	148	185	194	186	200	202	112	180	156	225	164	3,585
40	184	123	239	169	159	146	211	180	178	202	223	147	162	177	145	237	192	148	204	55	3,481
41	100	108	116	225	150	179	196	214	193	218	214	187	112	117	131	101	131	127	110	85	3,074
42	202	166	132	241	210	262	201	190	144	136	155	203	162	196	121	102	167	181	120	225	3,516
43	190	108	194	239	185	176	215	249	153	163	180	175	225	190	108	191	156	155	255	180	3,747
44	108	163	225	192	225	150	180	254	149	152	146	147	155	95	122	163	154	187	166	139	3,272
45	205	201	108	188	211	249	270	238	162	198	214	238	158	87	172	224	154	258	209	168	3,912
46	136	137	182	153	229	221	261	219	129	151	132	144	201	178	152	188	218	148	171	149	3,499
47	136	205	190	177	252	197	238	341	173	138	257	231	206	248	180	248	207	254	225	167	4,270
48	195	181	260	208	212	141	288	85	122	149	324	251	212	230	151	215	92	225	225	102	3,867
49	158	227	139	114	251	206	267	280	214	325	175	250	326	220	149	180	154	135	202	117	4,089
50	142	192	140	208	210	183	243	264	161	176	226	280	275	222	180	268	194	194	283	166	4,207
Total yield per row	4,914	5,530	5,528	6,361	6,781	6,139	7,740	7,738	6,420	7,511	7,405	7,610	7,533	7,475	7,611	7,104	7,153	7,587	7,986	5,859	137,985

NAVEL ORANGE (ANTELOPE HEIGHTS).—The navel-orange grove later referred to as the Antelope Heights navels is a plantation of 480 ten-year-old trees planted 22 by 22 feet, located at Naranjo, Cal. The individual tree records of this planting are shown in figure 2 and are the yields obtained in 1916. The general appearance of the trees gives a visual impression of uniformity greater than a comparison of the individual tree production substantiates; however, the distribution of the yields approximates closely the normal curve of errors, having a skewness of only 0.001 ± 0.037, as shown by figure 11.

FIG. 2.—Diagram showing the individual tree yield (in pounds) of the navel-orange grove (Antelope Heights).

NORTH

Yield per tree (pounds)

Row.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Total yield per row.
1	130	200	250	250	130	300	260	285	225	255	240	225	250	200	150	3,350
2	120	150	220	230	270	275	300	340	215	195	150	250	260	250	230	3,455
3	200	150	230	300	235	280	200	170	250	250	150	150	200	250	270	3,285
4	155	205	225	295	265	320	250	130	200	200	150	210	220	180	280	3,285
5	250	225	260	300	200	290	250	185	200	155	220	165	250	255	270	3,475
6	300	200	200	240	200	210	250	220	255	150	220	135	175	255	230	3,240
7	225	250	250	225	200	275	250	200	175	150	250	170	170	260	200	3,250
8	150	325	250	225	210	225	200	250	225	150	150	205	190	265	350	3,370
9	300	150	250	290	200	100	225	225	125	225	200	250	225	200	225	3,190
10	250	185	150	175	135	170	200	225	150	200	200	165	175	225	240	2,845
11	200	100	155	170	175	200	200	210	110	300	250	190	135	205	225	2,825
12	150	200	100	230	150	200	250	215	140	200	225	160	115	220	220	2,775
13	110	150	175	135	160	180	250	220	150	200	250	225	230	225	175	2,835
14	190	200	125	165	135	150	200	190	170	200	220	300	200	225	200	2,870
15	200	150	140	130	100	185	220	150	150	150	275	200	190	205	200	2,645
16	210	150	120	100	110	150	150	225	160	150	175	125	150	150	220	2,345
17	140	100	120	115	135	160	250	200	140	150	150	225	145	125	165	2,320
18	105	100	100	200	150	200	200	185	110	125	125	150	100	200	150	2,200
19	120	150	50	150	100	200	200	225	140	200	125	125	200	150	150	2,285
20	130	120	75	120	150	200	150	225	100	150	75	200	200	175	75	2,145
21	150	100	125	200	175	275	160	200	100	100	100	200	150	75	100	2,210
22	195	105	150	150	185	200	190	160	55	150	100	225	100	150	100	2,215
23	100	110	130	155	265	150	200	140	90	150	100	150	110	100	125	2,075
24	100	150	80	200	150	175	175	125	75	100	200	240	90	135	130	2,125
25	105	100	150	150	175	200	175	150	150	175	200	150	150	150	100	2,280
26	100	100	150	270	100	150	175	250	175	175	250	150	125	150	100	2,420
27	100	150	175	230	150	150	150	270	190	200	225	200	150	200	190	2,730
28	135	185	180	280	250	115	180	175	75	200	175	185	150	150	200	2,635
29	245	255	100	250	300	260	185	150	150	250	175	200	210	125	150	3,005
30	200	170	150	50	350	240	240	200	220	210	215	250	225	200	250	3,170
31	225	225	180	250	115	235	225	200	250	270	235	200	185	150	165	3,110
32	200	200	185	200	215	240	200	200	190	250	250	250	175	120	150	3,025
33	250	150	185	150	170	155	220	250	220	250	250	250	100	250	275	3,125
Total yield per row	5,740	5,460	5,385	6,580	6,010	6,815	6,930	6,745	5,330	6,235	6,275	6,475	5,700	6,175	6,260	92,115

VALENCIA ORANGE.—The Valencia orange grove is composed of 240 15-year-old trees, planted 21 feet 6 inches by 22 feet 6 inches, located at Villa Park, Cal. Figure 3 represents this planting and the individual tree yields which were obtained in 1916.

EUREKA LEMON.—The lemon yields were obtained from a grove of 364 23-year-old trees, located at Upland, Cal. Figure 4 represents the individual tree yields of this planting. The records extend from October 1, 1915, to October 1, 1916. The grove consists of 14 rows of 23-year-old trees, extending north and south, with 26 trees in a row, planted 24 by

FIG. 3.—Diagram showing the individual tree yield (in pounds) of the Valencia orange grove.

NORTHWEST

Yield per tree (pounds)

Row.	1	2	3	4	5	6	7	8	9	10	11	12	Total yield per row
1	100	375	250	275	275	150	200	175	225	275	200	125	2,625
2	375	325	275	375	200	375	225	230	275	225	225	262	3,367
3	37	75	175	260	150	300	25	275	450	250	325	250	2,572
4	63	175	250	350	300	275	325	300	250	275	275	275	3,113
5	75	75	175	300	300	325	300	275	375	275	200	275	2,950
6	100	212	125	25	200	275	325	250	250	100	150	75	2,087
7	50	136	100	75	250	100	250	100	190	150	225	225	1,851
8	300	200	100	75	150	200	200	275	200	200	150	250	2,200
9	100	175	50	300	263	363	350	300	337	338	375	150	3,101
10	100	150	100	150	150	262	250	275	363	337	350	250	2,737
11	75	125	125	275	125	275	150	225	312	188	225	150	2,250
12	225	225	350	250	300	150	300	300	262	265	225	225	3,077
13	425	200	225	325	75	275	425	275	375	300	25	225	3,150
14	50	350	200	125	225	150	225	250	250	300	300	175	2,600
15	437	238	225	200	350	375	225	225	300	400	375	350	3,700
16	400	163	150	300	450	300	400	350	350	250	325	225	3,663
17	350	275	400	300	462	288	412	363	375	300	350	100	3,975
18	175	325	250	350	350	350	100	300	300	200	175	175	3,140
19	350	350	400	250	275	275	212	188	350	250	150	100	3,150
20	400	350	450	375	200	300	350	375	275	350	325	100	3,850
Total yield per row	4,087	4,499	4,375	4,935	5,050	5,363	5,249	5,306	6,064	5,318	4,950	3,962	59,158

FIG. 4.—Diagram showing the individual tree yield (in pounds) of the Eureka lemon grove.

NORTH

Yield per tree (pounds)

Row.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Total yield per row.
1	259	258	188	251	379	325	201	232	349	237	296	264	241	104	3,584
2	204	157	227	314	381	264	283	211	292	329	273	218	236	222	3,611
3	475	235	305	183	412	251	210	271	443	348	378	298	310	222	4,341
4	383	292	274	263	288	356	260	172	327	322	345	295	279	375	4,231
5	510	250	190	202	313	300	157	169	327	399	281	304	321	280	4,003
6	421	188	277	338	369	346	286	140	382	346	233	245	313	333	4,217
7	425	228	167	195	343	363	202	167	334	377	312	300	308	266	3,987
8	218	274	206	133	234	341	211	169	386	429	298	281	277	100	3,557
9	301	257	176	276	230	312	167	206	180	471	342	238	339	342	3,837
10	409	264	164	188	221	319	234	149	439	354	248	279	293	287	3,848
11	152	165	123	194	276	336	278	177	314	441	283	281	282	279	3,581
12	79	118	150	120	258	222	261	245	268	308	278	322	306	222	3,157
13	287	196	170	264	292	241	235	159	341	399	317	307	298	295	3,801
14	348	258	161	267	345	345	237	216	380	353	365	301	200	285	4,061
15	81	199	144	180	268	278	213	209	336	312	299	279	223	248	3,269
16	250	146	106	282	253	290	245	218	360	368	322	338	344	245	3,767
17	111	165	223	259	227	361	278	116	304	315	338	318	326	311	3,652
18	195	162	135	241	275	291	256	122	259	363	385	358	356	294	3,692
19	333	156	170	154	357	325	186	80	226	335	369	358	223	288	3,560
20	291	172	127	203	104	239	222	112	364	371	349	402	276	338	3,570
21	111	189	181	255	332	328	235	176	298	398	329	307	204	244	3,587
22	361	114	127	296	326	252	396	158	282	325	294	322	287	236	3,776
23	210	245	184	315	414	294	201	128	416	313	326	410	222	276	3,954
24	140	197	245	207	350	388	343	289	410	302	341	283	253	202	4,040
25	126	229	216	185	275	297	412	241	328	262	333	280	131	277	3,792
26	241	263	189	220	397	399	341	335	438	266	413	237	328	316	4,383
Total yield per row	6,921	5,377	4,825	5,985	7,919	8,063	6,550	4,867	8,783	9,043	8,347	7,825	7,176	6,977	98,658

24 feet apart. This grove presents the most uniform appearance of any under consideration. The land is practically level, and the soil is apparently uniform in texture. The records show a grouping of several low-yielding trees; yet a field observation gives one the impression that the grove as a whole is remarkably uniform.

SEEDLING WALNUTS.—The walnut-tree records used in the following calculations were obtained during the seasons of 1915 and 1916 from a 24-year-old Santa Barbara softshell seedling grove, located at Whittier, Cal. The planting is laid out 10 trees wide and 32 trees long, entirely surrounded by additional walnut plantings, except on a part of one

FIG. 5.—Diagram showing the individual tree yield (in pounds) of the seedling walnut orchard.

NORTH

Yield per tree (pounds)

Row.	1	2	3	4	5	6	7	8	9	10	Total yield per row.
5	8	88	185	113	113	167	87	188	164	164	1,277
6	172	119	44	111	35	33	67	207	164	164	1,116
7	164	99	135	40	207	94	79	136	195	97	1,246
8	74	102	96	80	78	57	104	104	54	62	811
9	122	22	80	108	112	118	80	144	52	58	896
10	120	71	103	74	129	40	123	40	107	21	828
11	160	111	94	135	121	131	84	126	30	24	1,016
12	170	135	128	82	93	93	93	72	8	96	970
13	95	89	71	66	61	134	61	70	60	64	771
14	65	50	61	195	89	45	61	43	25	43	677
15	85	101	100	100	72	63	133	14	73	101	842
16	78	106	88	112	76	86	15	113	53	83	815
17	51	34	101	71	82	99	15	81	28	50	612
18	17	104	25	95	57	76	96	69	25	65	629
19	75	69	73	68	6	35	90	80	94	13	603
20	117	179	123	35	47	51	104	85	42	75	858
21	91	188	88	105	65	86	91	31	72	97	914
22	122	122	103	180	120	165	144	106	85	105	1,252
23	99	144	69	124	48	197	122	77	117	83	1,080
24	83	155	170	32	142	97	101	160	90	87	1,117
25	44	83	198	192	113	76	81	131	65	39	1,022
26	86	176	125	130	147	119	210	113	149	131	1,386
27	71	50	151	58	112	71	104	94	113	126	950
28	87	111	140	170	123	96	160	109	25	148	1,169
29	41	136	74	54	115	99	130	87	166	129	1,031
30	86	158	95	80	75	178	90	115	150	128	1,155
31	169	150	151	138	148	151	73	94	166	235	1,475
32	112	100	166	188	39	192	157	215	160	201	1,538
Total yield per row	2,664	3,052	3,037	2,936	2,625	2,849	2,755	2,904	2,545	2,689	28,056

side which is adjacent to an orange grove. The trees are planted on the square system, 50 feet apart. Figure 5 gives the yield and arrangement of these trees.

JONATHAN APPLES.—The apple records [1] were obtained from a 10-year old Jonathan apple orchard located at Providence, Utah. The surface soil of this orchard is very uniform to all appearances except on the extreme eastern edge, where the percentage of gravel increases slightly. The trees are planted 16 feet apart, east and west, and 30 feet apart north and south. Figure 6 gives the yield and arrangement of these trees.

[1] The authors wish hereby to express their appreciation of the kindness of the Utah Experiment Station in furnishing these records.

RANGE OF INDIVIDUAL TREE YIELDS

The extremes of individual tree productivity are shown in Table I. This indicates a wide range of variation even in oranges, lemons, and apples, which are clonal varieties. The greatest range, however, is in the yield of the seedling walnuts. The variations set forth in this table may seem excessive to workers with annual crops. To those familiar with the variation in tree crops, however, this will be recognized as only the normal variation which occurs in most fruit plantations growing on apparently uniform soil, as mentioned in a previous section. Wide variation in the vigor of the rootstock, as well as variation in soil productivity, may have been instrumental in causing such a variation in yield.

FIG. 6.—Diagram showing the individual tree yield (in pounds) of the Johnathan apple orchard.

Yield per tree (pounds)

Row.	8	7	6	5	4	3	2	1	Total yield per row.
37	387	250	550	362	212	437	337	212	2,747
38	187	200	175	400	350	100	500	412	2,324
39	337	112	575	550	300	550	475	262	3,161
40	425	437	400	450	250	362	287	437	3,048
41	425	450	425	375	525	375	437	375	3,387
42	100	212	350	575	337	137	337	300	2,348
43	325	437	337	375	500	550	350	362	3,236
44	125	350	262	437	212	325	475	375	2,561
45	375	400	350	412	550	450	437	350	3,324
46	300	450	312	250	525	337	437	337	2,948
47	512	487	212	325	425	500	437	300	3,198
48	162	312	250	37	537	300	375	250	2,223
49	275	375	50	286	475	275	412	225	2,087
50	175	462	362	387	187	337	437	212	2,559
51	312	387	312	350	262	337	500	475	2,935
52	125	400	375	150	375	375	50	300	2,150
53	300	375	250	312	175	375	450	337	2,574
54	250	200	250	250	75	300	262	75	1,662
55	350	175	450	261	287	87	312	100	2,548
56	187	150	50	350	375	100	275	425	1,912
57	300	300	262	375	325	425	350	225	2,562
58	200	187	387	362	425	200	312	375	2,448
59	250	100	337	362	312	100	225	187	1,873
60	187	200	337	337	450	300	200	225	2,236
61	112	287	175	325	325	200	150	150	1,724
62	50	125	200	375	437	275	275	125	1,862
63	187	100	287	50	87	150	325	287	1,473
64	162	150	187	200	50	75	100	250	1,174
Total yield per row	7,082	8,070	8,469	9,520	9,345	8,334	9,519	7,945	68,284

TABLE I.—*Range of variability in crop production of fruit and nut trees*

[Extremes and range expressed as percentages of the mean yields of the respective plantations]

Kind of fruit.	Mean yield.	Extreme yield.	Range.	Mode.	Skewness.
	Pounds.	*Per cent.*	*Per cent.*	*Pounds.*	
Naval oranges (Arlington)	137 ± 1.6	9.1 to 246.3	237.2	129.8	0.143 ± 0.025
Naval oranges (Antelope Heights)	186 ± 1.7	33.4 to 193.8	160.4	186.1	.001 ± .037
Valencia oranges	246 ± 4.3	5.1 to 188.0	182.9	270.8	− .250 ± .053
Eureka lemons	270 ± 2.9	32.4 to 180.3	147.9	299.9	− .359 ± .043
Seedling walnuts	86 ± 1.6	14.4 to 276.1	261.7	75.6	.269 ± .049
Jonathan apples	304 ± 5.6	12.3 to 193.2	180.9	345.1	− .329 ± .055

The biometrical constants for the several plantations are given in Table II. The oranges, lemons, and apples, as might be expected, show less variability than the seedling walnuts. The coefficient of variability of the clonal varieties ranges from 29.72 to 41.23 per cent. This total range of only 11.51 per cent shows a marked similarity of the extent of variation.

TABLE II.—*Variability in yield of the different individual fruit trees*

Kind of fruit.	Crop.	Acreage per tree.	Total number of trees.	Mean yield per tree.	Standard deviation.	Coefficient of variability.	Probable error.	
							Pounds per tree.	Percentage of mean.
				Pounds.	*Pounds.*			
Naval orange (Arlington)	1915	0.011	1,000	137.6±1.2	54.42±0.82	39.55±0.68	37	26.67
Naval orange (Antelope Heights)	1916	.011	495	186.2±1.7	55.33±1.19	29.72±0.69	37	20.05
Valencia orange	1915	.011	240	246.3±4.3	97.84±3.01	39.72±1.40	66	26.79
Eureka lemon	1915	.013	364	270.7±2.9	81.38±2.03	30.06±0.81	55	20.28
Seedling walnut	1915	.057	280	99.8±1.9	47.77±1.36	47.86±1.64	32	32.28
Seedling walnut	1916	.057	280	77.6±1.7	41.94±1.19	53.91±1.92	28	36.36
Seedling walnut, average	1915 and 1916	.057	280	86.4±1.6	40.10±1.14	46.41±1.58	27	31.30
Jonathan apple	1914	.011	224	303.9±5.6	125.30±4.00	41.23±1.52	85	27.81

The probable error, expressed in pounds of fruit per tree, is the greatest in case of the Jonathan apple, amounting to 85 pounds, while the Valencia orange and the Eureka lemon fall to 66 and 55 pounds, respectively. Such probable errors, expressed in pounds per tree, are not comparable, however, unless the mean yields are approximately the same. The probable error expressed as a percentage of the mean is therefore added to Table II to make it more easily compared with tables of other writers who have seen fit to use this constant rather than the coefficient of variability.

METHODS OF CALCULATING VARIABILITY

The yields of the various fruit plantations have been studied, with trees singly and combined into plots of various sizes. The coefficient of variability and probable error have been used as the basis of comparison in most cases.

Plots of different sizes necessarily have varying mean yields per plot; therefore the coefficient of variability is more readily interpreted than the standard deviation. The probable error may only be used with accuracy in cases where the number of variants is relatively large and their distributions normal.

VARIABILITY OF ORCHARD PLOT YIELDS

EFFECT OF INCREASING THE NUMBER OF ADJACENT TREES PER PLOT

The first point studied was the effect of increasing the number of adjacent trees per plot, measured by the coefficient of variability. Based on the theory of random sampling of variables, the average production of 10, or even 5, trees should be a more typical sample of the orchard than that

of 1 tree. The reduction of the coefficient of variability by combining a number of adjacent trees in a plot would, however, be expected to fall short of the theoretical reduction, because such a combination may have a tendency to group trees of similar productivity together. Gradual soil variation from one side of the plantation to the other, or irregularities of the field which are larger than the area taken up by a single tree, will tend to bring about a correlation between the yields of adjacent trees.

For practical purposes the two more or less antagonistic sources of variation between plots may be arranged in two groups:

1. Those which may cause the variations to become greater as the size of the plot increases—for example, variation in soil productivity.

2. Those which may cause the variations to become less as the size of the plot increases—for example, variations in inherent productiveness of the trees. This may depend to no small degree on the variation in vigor and character of growth of the rootstock. Measured by crop production, it may be practically impossible with grafted or budded trees to separate by mere inspection the variation which may be caused in inherent qualities of the bud from those of the rootstock on which it is propagated.

The reduction of the coefficient of variability in the several plantations as a result of increasing the number of adjacent trees per plot is shown in Table III. The acreage per plot is recorded for sake of comparison with similar work by agronomists, where the size of the plots studied has been dependent entirely on acreage rather than number of plants to the plot. Other biometrical constants are likewise included for ease of comparison with above-mentioned studies.

The effect of increasing the number of adjacent trees per plot on reducing the coefficient of variability between the plots of all the fruit crops studied is shown in the summary of Table III, and figure 7 shows the same thing graphically. The curves show a marked similarity between the varieties of fruits and agree quite closely in demonstrating that there is little to be gained in including more than eight adjacent trees in a plot. As a rule, there is a rapid reduction in the coefficient, as progress is made from a 1-tree to an 8-tree plot. Increasing the plot above eight adjacent trees shows only a comparatively small reduction of the coefficient of variability. In fact, the reduction is not significant when the probable errors[1] are considered. The Antelope navels and apples show a reduction slightly less than the probable error between a 4-tree and an 8-tree plot. Again, the lemons show an apparently exceptional reduction when the 16-tree plot is compared with the 24-tree plot. The same is true of the 8- and 16-tree plots of apple trees. These exceptions are in part explained, where they concern the larger plots,

[1] The probable error of the difference between two averages A_1 and A_2, of which the probable errors E_1 and E_2 are known, is the square root of the sum of the squared probable errors; or probable difference of $A_1 - A_2 = \pm\sqrt{E_1^2 + E_2^2}$

by the fact that in a given area, as the size of the plot increases the number of plots necessarily decreases, and thus lessens the reliability of the comparisons. Thus, these exceptions may be due to chance in a small population and might not hold true with a larger number of variants.

TABLE III.—*Effect of increasing the number of adjacent trees per plot*

Kind of tree.	Number of trees per plot.	Number of plots.	Acreage per plot.	Mean yield per plot.	Standard deviation.	Coefficient of variability.	Probable error.	
							Pounds per tree.	Percentage of mean.
				Pounds.	*Pounds.*			
Navel oranges (Arlington)	1	1,000	0.011	137.6± 1.2	54.42± 0.82	39.55±0.68	37	26.67
Do	2	500	.022	275.6± 2.9	95.60± 2.03	34.68±0.82	32	23.39
Do	4	250	.044	551.6± 7.4	173.89± 5.24	31.52±1.05	29	21.26
Do	8	125	.088	1,100.8± 19.2	319.00± 13.60	28.98±1.30	27	19.55
Do	16	60	.176	2,220.0± 56.0	642.60± 39.58	28.95±1.92	27	19.53
Do	24	40	.264	3,343.8± 92.5	867.70± 65.43	25.95±2.08	24	17.50
Navel oranges (Antelope Heights)	1	495	.011	186.2± 1.7	55.33± 1.19	29.72±0.69	37	20.05
Do	2	247	.022	373.0± 4.2	97.21± 2.95	26.06±0.84	33	17.58
Do	4	125	.044	742.7± 10.0	164.37± 7.07	22.13±1.00	28	14.93
Do	8	61	.088	1,460.7± 26.7	309.05± 18.90	21.16±1.35	26	14.27
Do	16	30	.176	2,973.3± 68.4	555.55± 48.38	18.68±1.68	23	12.60
Do	24	20	.264	4,450.0±121.6	806.54± 86.01	18.10±1.91	23	12.21
Valencia oranges	1	240	.011	246.3 ± 4.3	97.84± 3.01	39.72±1.40	66	26.79
Do	2	120	.022	487.92± 10.0	161.80± 7.04	33.16±1.61	55	22.37
Do	4	60	.044	991.7 ± 23.9	275.20± 16.92	27.75±1.84	46	18.72
Do	8	30	.088	1,966.7 ± 59.4	482.60± 42.02	24.54±2.26	41	16.55
Do	16	15	.176	3,813.3 ±158.6	910.58±112.13	23.88±3.10	38	16.11
Do	24	10	.264	5,880.0 ±243.0	1,140.00±171.90	19.39±3.03	32	13.08
Eureka lemons	1	364	.013	270.7 ± 2.9	81.38± 2.03	30.06±0.81	55	20.28
Do	2	182	.026	544.3 ± 6.8	135.40± 4.78	24.88±0.93	46	16.78
Do	4	91	.053	1,081.0 ± 17.3	245.35± 12.27	22.70±1.19	41	15.31
Do	8	42	.106	2,172.0 ± 46.7	448.50± 33.01	20.65±1.57	38	13.93
Do	16	21	.211	4,395.0 ±121.7	827.00± 86.07	18.82±2.02	35	12.69
Do	24	13	.317	6,692.0 ±153.5	820.30±108.50	12.26±1.65	23	8.27
Seedling walnuts	1	280	.057	86.4 ± 1.6	40.10± 1.14	46.41±1.58	27	31.30
Do	2	140	.114	178.7 ± 3.6	62.40± 2.52	34.92±1.57	21	23.55
Do	4	70	.228	353.6 ± 8.6	106.40± 6.06	30.09±1.86	18	20.30
Do	8	35	.456	717.1 ± 20.7	181.24± 14.60	25.27±2.16	16	17.04
Do	16	17	.912	1,409.4 ± 58.0	354.40± 41.00	25.15±3.07	15	16.96
Do	24	11	1.368	2,154.6 ±103.3	508.00± 73.05	23.58±3.53	14	15.90
Jonathan apples	1	224	.011	303.9 ± 5.6	125.30± 4.00	41.23±1.52	85	27.81
Do	2	112	.022	609.8 ± 12.3	193.00± 10.44	31.65±1.87	65	21.35
Do	4	56	.044	1,210.7 ± 28.8	319.40± 20.40	26.38±1.80	54	17.79
Do	8	28	.088	2,414.3 ± 76.9	603.38± 54.38	24.99±2.39	51	16.86
Do	16	14	.176	4,864.3 ±175.5	973.68±124.11	20.02±2.65	41	13.50
Do	24	9	.264	7,277.8 ±313.9	1,396.00±221.90	19.18±3.16	35	12.94

SUMMARY

Number of trees per plot.	Coefficient of variability.							Average reduction of coefficient of variability by increasing number of adjacent trees per plot.	
	Navel oranges.		Valencia oranges.	Eureka lemons.	Seedling walnuts.	Jonathan apples.	Average.	Increase from—	Average reduction.
	Arlington.	Antelope Heights.							
1	39.55±0.68	29.72±0.69	39.72±1.40	30.06±0.81	46.41±1.58	41.23±1.52	37.78±0.52	......	....
2	34.68±0.82	26.06±0.84	33.16±1.61	24.88±0.93	34.92±1.57	31.65±1.87	30.89±0.55	1 to 2	6.89±0.76
4	31.52±1.05	22.13±1.00	27.75±1.84	22.70±1.19	30.09±1.86	26.38±1.80	26.76±0.62	2 to 4	4.13±0.83
8	28.98±1.30	21.16±1.35	24.54±2.26	20.65±1.57	25.27±2.16	24.99±2.39	24.27±0.77	4 to 8	2.49±0.99
16	28.95±1.92	18.68±1.68	23.88±3.10	18.82±2.02	25.15±3.07	20.02±2.65	22.58±1.01	8 to 16	1.69±1.27
24	25.95±2.08	18.10±1.91	19.39±3.03	12.26±1.65	23.58±3.53	19.18±3.16	19.74±1.08	16 to 24	2.84±1.48

The averages of all six fruit plantations show that there is a rapid reduction of the coefficient of variability until the 8-tree plot is reached, but from then on the reduction is less in comparison with the probable error. (See Table III, summary, and figure 8.)

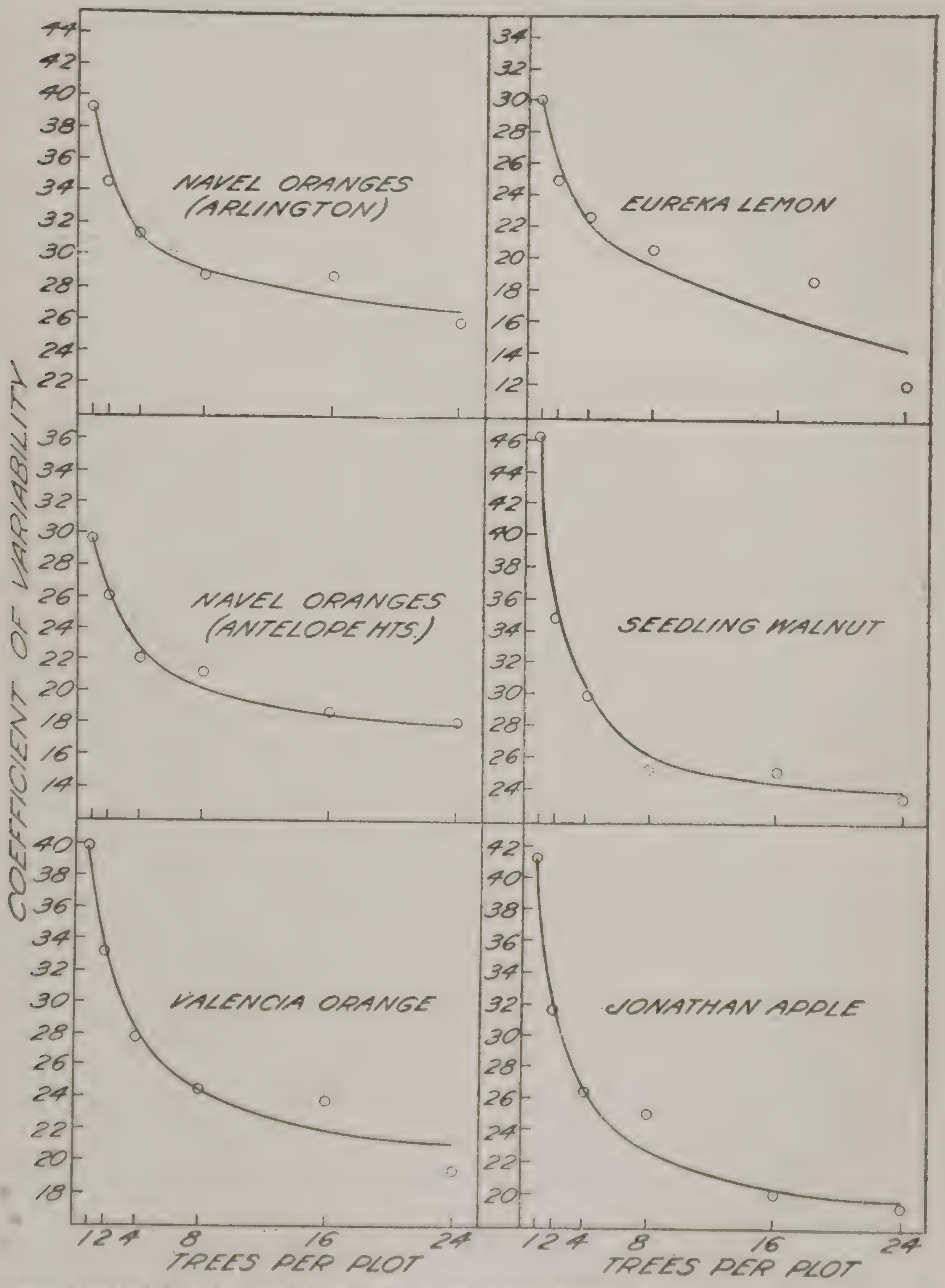

FIG. 7.—Graphs of the reduction of the coefficient of variability by increasing the number of adjacent trees to the plot.

Although the average reduction of the coefficient between the 8-and 16-tree plots is doubtful when compared with the probable error, this reduction in all six cases is constant—that is, the variation occurs in

one direction only, and therefore has more significance than is indicated by a mere comparison of the averages.

EFFECT OF SYSTEMATIC DISTRIBUTION OF PLOTS OVER THE AREA STUDIED

The importance of distributing plots over the experimental area is more or less obvious, and has been dwelt upon by many writers. Its value arises from the fact that the soil varies over the area, and it is better to have similar-sized plots on both high- and low-yielding areas than to have them solely on one or the other kind of soil. The method should be of special value on areas which vary rather uniformly in one direction.

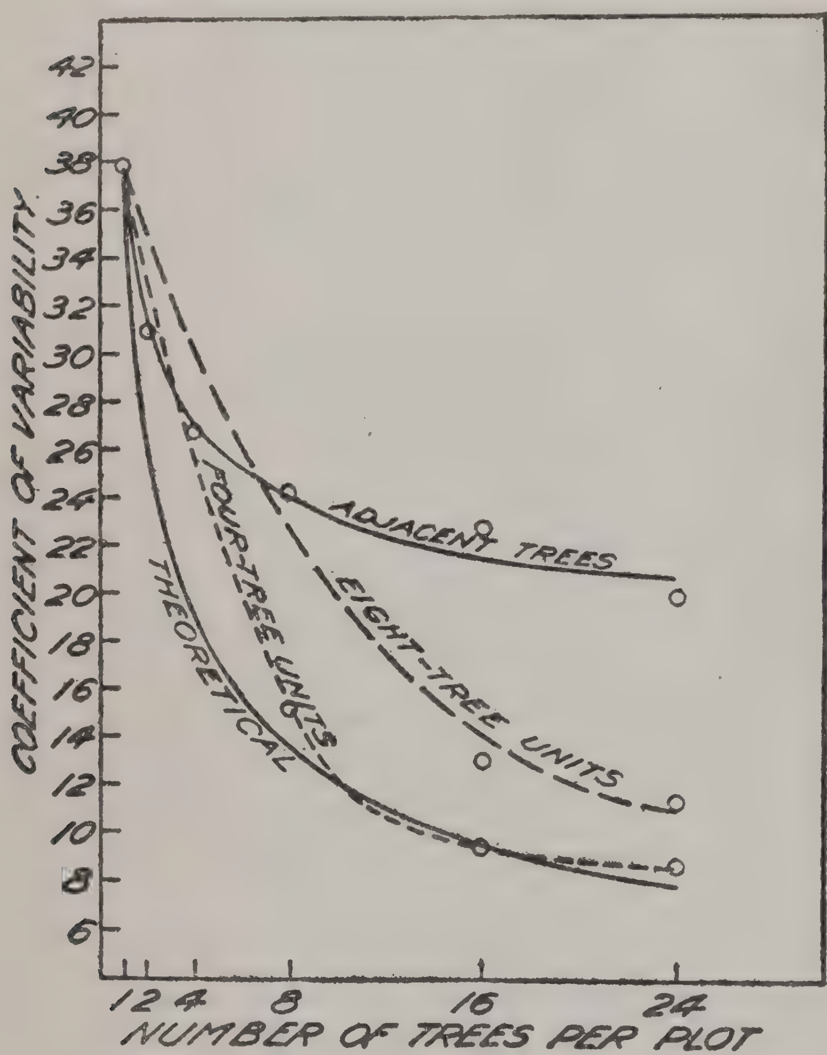

FIG. 8.—Graphs of the reduction of the coefficient of variability by increasing the number of trees to the plot.

Increasing the number of trees to the plot in scattered units of either four or eight trees gives a more typical sample of the productivity of the total planting than the same number of adjacent trees. In scattering the plots throughout the area studied, they were systematically repeated. For example, if there were 100 plots in all to be grouped in pairs, the first and fifty-first, and the second and fifty-second were united, and so on through the series. If a quadruple series was desired, the first, twenty-sixth, fifty-first, and seventy-sixth plots were combined.

Table IV shows the results of scattering 4- and 8-tree plots, respectively, in the plantations studied. Figure 8 illustrates the reduction of the coefficient of variability by increasing the number of trees to the plot in both 4- and 8-tree scattered units, compared with the average coefficient of variability for the several fruits by increasing the size of a plot from 1 to 24 adjacent trees, together with the theoretical curve calculated from the mean coefficient of variability of all the 1-tree units.

A comparison of the curve for adjacent trees and those for scattered units shows at once the marked decrease in favor of the scattered units.

TABLE IV.—*Effect of increasing number of trees per plot in scattered units*

FOUR TREES IN A UNIT

Unit No.	Total number of trees per plot.	Coefficient of variability.						
		Navel oranges.		Valencia oranges.	Eureka lemons.	Seedling walnuts.	Jonathan apples.	Average.
		Arlington.	Antelope Heights.					
1....	4	31.52±1.00	22.13±1.00	27.75±1.80	22.70±1.10	30.09±1.85	26.38±1.80	26.76±0.60
2....	8	18.59±0.82	13.06±0.85	16.04±1.43	14.76±1.07	12.36±1.01	15.89±1.47	15.12±0.47
3....	12	15.11±0.81	11.07±0.88	16.48±1.81	12.61±1.16	14.17±1.41	12.04±1.37	13.58±0.53
4....	16	10.76±0.66	9.52±0.87	10.39±1.29	8.01±0.81	8.59±0.99	8.44±1.08	9.29±0.40
5....	20	11.15±0.76	5.74±0.58	9.28±1.28	9.48±1.07	6.97±0.89	7.75±1.13	8.40±0.40
6....	24	9.89±0.74	6.38±0.72	13.16±2.02	5.38±0.66	10.05±1.45	6.12±0.97	8.49±0.49

EIGHT TREES IN A UNIT

Unit No.	Total number of trees per plot.	Arlington.	Antelope Heights.	Valencia oranges.	Eureka lemons.	Seedling walnuts.	Jonathan apples.	Average.
1....	8	28.98±1.30	21.16±1.35	24.54±2.26	20.65±1.57	25.27±2.16	24.99±2.39	24.27±0.77
2....	16	17.49±1.09	9.21±0.83	15.22±1.92	10.98±1.17	10.94±1.27	13.20±1.69	12.84±0.56
3....	24	11.78±0.89	7.44±0.84	14.34±2.21	11.70±1.44	11.35±1.63	10.99±1.75	11.27±0.63
4....	32	14.85±1.30	8.87±1.14	8.72±1.57	7.56±1.16	6.68±1.13	10.54±1.90	9.54±0.57
5....	40	10.09±0.96	8.60±1.24	5.24±1.02	7.81±1.32	11.01±2.01	4.94±1.05	7.95±0.54

The 4-tree unit apparently gives a greater degree of accuracy than the 8-tree unit with the same total number of trees. This point is clearly shown by the curves. In considering a total number of either 8, 16, or 24 trees the curve for the 4-tree unit approaches more nearly the theoretical curve than the curve of the 8-tree unit. With a total number of 24 trees, for example, the 4-tree units would be scattered about regularly in six different places, while the 8-tree units would be located in three different places. The larger number of unit plots thus gives a more typical sample of the area than half as many units with twice the number of trees in a unit.

In combining both the 4- and the 8-tree units in the regular scattering of ultimate plots, an attempt was made to throw both high- and low-yielding small plots into a combination, although a systematic distribution was maintained. The fact that the curve of the 4-tree units drops below the theoretical in one place indicates that this attempt was successful. An ordinary regular scattering of the ultimate plots might not approach the theoretical curve of random sampling so closely if a knowledge of the relative productivity of the soil was not available before arranging the plots. A 4-tree unit might not be practical in tests of cultural methods, fertilizer, or irrigation trials. In such trials an 8-tree plot is usually the smallest practical unit. In the case of walnuts, however, which should be planted at least 50 by 50 feet, a 4-tree unit in a single row would allow for a space 50 feet wide by 200 feet long, and, if guard rows were planted between the experimental trees, the plots would be 100 by 250 feet, a very practical size upon which to handle orchard machinery. For the trial of rootstocks, pruning experiments, variety tests, etc., the 4-tree plot is a practical-sized

unit and could be expected to give more reliable results if repeated at four regularly-placed intervals than either two 8-tree units, or 16 adjacent trees—that is, such a regular scattering of the several units which make up the combination plot reduces the error of the final comparisons which is caused by the variation in soil productivity.

The fact that marked soil variations occur which tend to make adjacent trees or adjacent plots yield alike, even on soils which were chosen because of their apparent uniformity, is well shown by the work of Harris (*1915*). The criterion for the measurement of such variability proposed by this author is the coefficient of correlation between neighboring plots of the field.[1] Applying this to the Arlington navel oranges, the writers have calculated the correlation between the yield of the 8-tree plot as the ultimate unit, and the yield of the combination of four such adjacent plots and it was found that

$$r = +0.533 \pm 0.085.$$

This shows a marked correlation, indicating a pronounced heterogeneity in the soil of this grove, influencing fruit production.

However, when we calculate the correlation between the 8-tree plot as the ultimate unit and the yield of the combination of four such systematically scattered plots, it is found that—

$$r = +0.137 \pm 0.120$$

This coefficient is practically equal to its probable error and can be regarded as significantly zero. This is merely another means of calculating the value of scattering a 32-tree plot in four ultimate plots of 8 trees each rather than selecting 32 adjacent trees.

DEGREE OF ACCURACY EXPECTED WITH A PLOT OF A GIVEN SIZE

Assuming, for example, that experimental plots have been laid out in the navel oranges (Arlington) with a total of 32 trees to the plot in four scattered units of eight trees each, the question might logically be asked, "What differences in the yields of such plots can safely be attributed to differential treatment as different methods of irrigation or fertilization, and what may probably be due to mere chance because of soil heterogeneity and the fluctuating variation of the trees?"

Table IV shows a coefficient of variability of 14.85 ± 1.30 in this plantation laid out in 32-tree plots of four scattered units of eight trees each. The probable error,[2] then, in this example, that such a plot of 32 trees is typical of the area in question, is $14.85 \times 0.6745 = \pm 10.02$

[1] The formula used is

$$r_{p_1 p_2} = \frac{\{[S(C_p^2) - S(p^2)]/m[n(n-1)]\} - \bar{p}^2}{s_p^2}$$

where p = yield of an individual plot; m = number of larger plots, each made up of n contiguous ultimate units; C_p = yield of the larger combination plots; S = summation of the yields of all the ultimate or combination plots of the field.

[2] The probable error of a single variant of a population may be defined as that departure from the mean on either side, within which exactly one-half of the variants are found. Expressed as a percentage of the mean, it is determined by multiplying the coefficient of variability by 0.6745.

per cent of the mean production. That is, the chances are even that any plot as described, of 32 trees, will fall within ± 10.02 per cent above or below the true mean. The chances are equally as good that such a plot will not fall within the accuracy of ± 10.02 per cent of the mean. In comparing two such plots, both with the same probable error of ± 10.02 per cent, the probable error of such a comparison will be greater than the probable error of one—that is, it will be equal to ± 10.02 per cent $\times \sqrt{2} = \pm 14.17$ per cent. Therefore, if plots undergoing differential treatment vary from each other by only ± 14.17 per cent of the mean of the plantation, half the time such differences in yield may be due to the treatment, and half the time they may be due to casual variation.

It is clear, then, if fertilizer or irrigation experiments laid out in such plots differ from each other by only 14.17 per cent of the mean production of the total area, we are not assured beyond an even chance that the difference is a real difference due to the factors which are being experimented upon. So slight an assurance can hardly be expected to be sufficiently reliable to prompt a farmer to purchase fertilizer, to change his method of irrigation, or to undertake any new business; much less will this assurance justify an experimenter in drawing conclusions from the result of a field trial.

Our present knowledge of orchard fertilization in the arid West will hardly justify any assumption on our part more reliable than an even chance that one fertilizer will produce an increased yield of fruit compared with another, or even cause an increase over an untreated plot. The same thing may be said in comparing different methods of irrigation. In most cultural trials we would therefore be comparing two results where the difference may occur in either direction. (Tables V and VI.)[1]

TABLE V.—*Table of odds for differences which may occur in either direction*

Difference from the mean in terms of probable error.	Difference between two results in terms of probable error of each result.	Odds against such difference occurring under uniform conditions.
1.00	1.41	1 to 1
1.25	1.76	3 to 2
1.44	2.03	2 to 1
1.71	2.41	3 to 1
1.90	2.68	4 to 1
2.00	2.83	9 to 2
2.05	2.87	5 to 1
2.50	3.53	10 to 1
2.93	4.13	20 to 1
3.00	4.24	22 to 1
3.20	4.51	30 to 1
4.00	5.66	140 to 1
4.90	6.93	1,000 to 1
5.00	7.07	1,350 to 1

[1] Tables V and VI are taken from the writings of Wood (*1911*) who in turn adopted them "from one of the standard reference books on astronomy."

TABLE VI.—*Table of odds for differences which may occur in one direction only*

Difference from the mean in terms of probable error.	Difference between two results in terms of probable error of each result.	Odds against such difference occurring under uniform conditions.
1.00	1.41	3 to 1
1.25	1.76	4 to 1
1.44	2.03	5 to 1
1.58	2.23	6 to 1
1.71	2.41	7 to 1
1.81	2.55	8 to 1
1.90	2.68	9 to 1
2.00	2.83	10 to 1
2.48	3.50	20 to 1
2.70	3.81	30 to 1
2.89	4.07	40 to 1
3.00	4.24	44 to 1
3.03	4.28	50 to 1
3.44	4.85	100 to 1
4.00	5.66	290 to 1
5.00	7.07	2,700 to 1

On assuming that a 10-to-1 chance is a reasonable assurance, the question logically arises, "What difference between any two plots must be manifested for this degree of confidence that the difference is due to treatments applied?" By referring to columns 2 and 3, Table V, one may find the difference in terms of the probable error which is necessary between two results to obtain this degree of reliability. Here it can be seen in column 2 that there must be a difference 3.53 times the probable error to give the odds of 10 to 1 in column 3 against such a difference occurring under uniform conditions. Thus we find in this example that the difference between two 32-tree plots in four scattered units must be at least 14.17 per cent $\times$ 3.53 = 50.02 per cent of the mean production, to give the assurance of a 10-to-1 chance that the difference is due to fertilizer, irrigation, or whatever factors are under consideration.[1] Even with this difference, conclusions based on such results obtained in this navel orange (Arlington) grove may be correct 10 times out of 11 and wrong once out of 11 times.

On turning now to the 32-tree plot of adjacent trees with navel oranges (Arlington), it is seen the probable error is 16.42 per cent. To proceed as before, 16.42 per cent, the probable error of one plot, $\times\sqrt{2}$ = 23.22 per cent, the probable error of the difference; 23.22 $\times$ 3.53 = 81.97 per cent. Therefore a difference between two such plots of 81.97 per cent of the mean of the total area would be necessary to give the assurance that such differences are real 10 times out of 11 and due to pure chance

[1] If comparisons were being made between two radically different treatments which were known to produce different effects in fruit production, such as irrigation compared with dry farming, or the use of large quantities of stable manure on light soils, compared with no manuring, then reference should have been made to columns 2 and 3 in Table VI, which are based on the differences occurring in one direction only.

only once out of 11 times; yet this grove was chosen because of its apparent regularity, for it has been judged sufficiently uniform for plot trials.

The point might be justly raised that the small number of plots involved in the above calculations are not sufficient to give the laws of chance a fair opportunity of asserting themselves. On laying the area out in plots of adjacent trees there were 30 plots, while made up of scattered units there were 31 plots. Figure 9 shows the distribution of these plots, together with the theoretical curve, which was caclulated for the scattered-unit curve. The scattered-unit curve closely approaches the theoretical normal curve of errors, and therefore reliance can be placed upon its probable error.

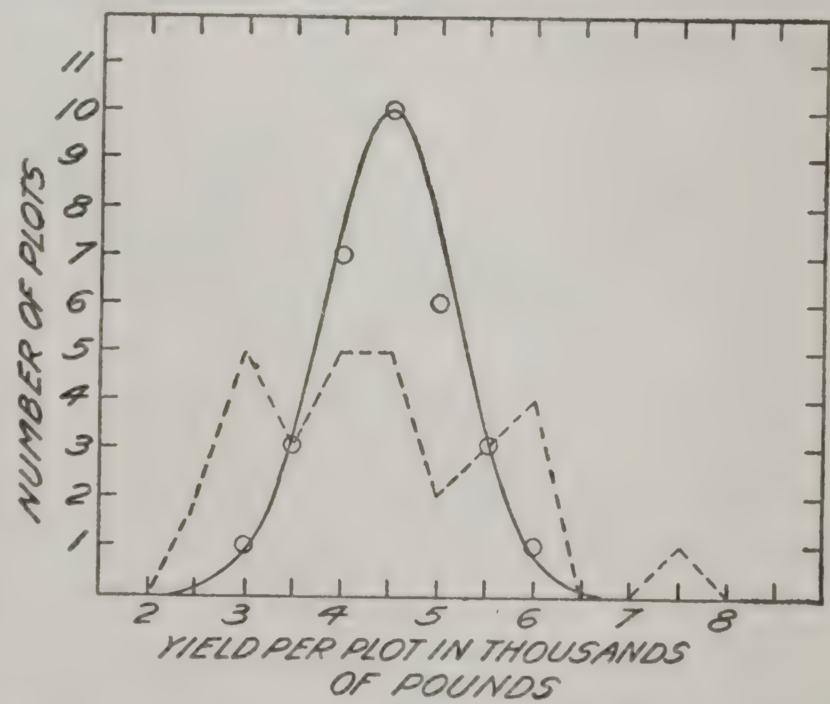

FIG. 9.—Graphs of production, 32-tree plot, navel oranges (Arlington).
——— Scattered in four 8-tree units.
- - - - Adjacent 32-tree units.

In the case of the plots of adjacent trees, however, the 30 units are not sufficient to give the laws of chance fair play. Table VII sums up the results of the foregoing calculations, adding extreme and mean yields of the two different types of plots and the theoretical probable error. The theoretical probable error based on the theory of random sampling for a hypothetical 32-tree plot is the probable error of one tree $26.67 \div \sqrt{32} = 4.71$ per cent. This is readily calculated from the distribution of the yields on a one-tree unit, the curve of which is shown by figure 10. The large number of trees involved, even though the distribution is not normal, justifies the use of the probable error as a minimum probable error. Based upon the theory of random sampling, two hypothetical 32-tree plots with a probable error of 4.71 per cent should show a minimum difference of $(4.71 \times \sqrt{2} \times 3.53) = 23.51$ per cent to give an assurance of a 10-to-1 chance that such a difference is real and not due to casual variation. Therefore, if the calculations in Table VII based on adjacent trees can not be fully relied upon because of the small number (30) in the population and because their distribution is not normal, we may at least reasonably expect that the necessary difference between two such plots will fall between the theoretical 23.51 per cent and 81.97 with a practical certainty that it will be greater than 50.02 per cent of the mean.

TABLE VII.—*Comparison of the reliability of a plot of 32 adjacent trees with that of a 32-tree plot of four scattered units of 8 trees each. Navel oranges (Arlington)*

Plot.	Extreme yields per plot.	Mean yield per plot.	Standard deviation.	Probable error per plot (percentage of mean).	Difference necessary to give 10-to-1 assurance.	
					Percentage of the mean.	Observed yield.
	Pounds.	*Pounds.*	*Pounds.*			*Pounds.*
Adjacent trees	2,500 to 7,500	4,367±131	1,063±93	±16.42	±81.97	±3,580
Scattered-unit plot	3,000 to 6,000	4,484± 81	666±57	±10.02	±50.02	±2,243
Theoretical (based on random sampling)				±4.71	±23.51	±1,035

On turning now to the navel oranges (Antelope Heights), which the calculations show to be the most uniform planting of any observed, the question might arise as to what degree of reliability may be expected in comparing two 16-tree plots. Table VIII shows a comparison between such plots made up of adjacent trees and plots composed of scattered units of four trees each together with the theoretical. A much greater range is found with the plot of adjacent trees, while the necessary difference between two plots for a 10-to-1 assurance is 62.94 per cent. With plots made up of scattered units the necessary difference is 32.05 per cent. The heterogeneity of this soil is clearly shown by both the increase in range and the increased probable error when plots are composed of adjacent trees. This block of trees, however, appeals to the observer as unusually uniform and would be considered desirable for plot experimentation, the fluctuation in the productivity of the trees approaching closely

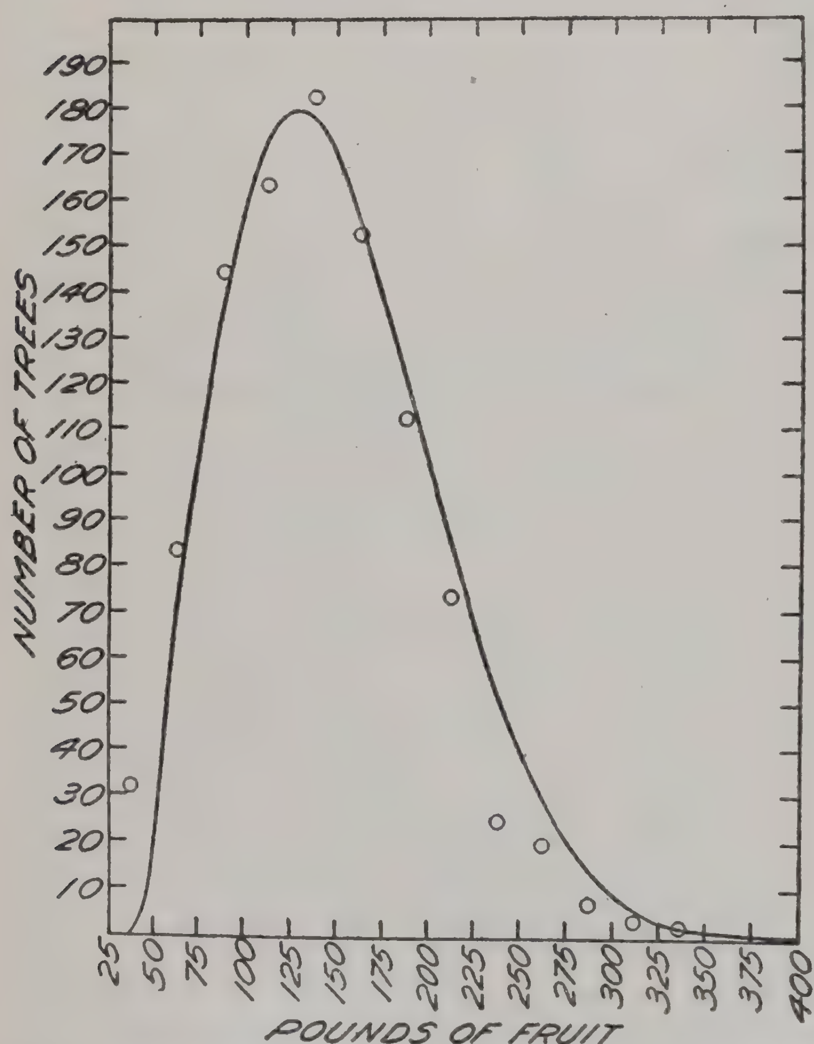

FIG. 10.—Curve of yields of individual trees, navel orange (Arlington).

the normal curve (see fig. 11); the mean production per tree is 186.2 ± 1.7 pounds, standard deviation 55.33 ± 1.19, coefficient of variability 29.72 ± 0.69 per cent, probable error 20.05 per cent of the mean, skewness 0.001 ± 0.037. Nevertheless, if devoted to plot experiments, a difference between two plots of 16 adjacent trees each, of even 62.94 per cent of the mean production, might be due to differential treatment 10 times out of 11 and due to casual variations of soil and trees once out of 11. The calculations sound a note of warning against drawing conclusions between such plots if the differences are less than 50 per cent of the mean production of the plantation, provided we wish to have such conclusions as dependable as a 10-to-1 chance.

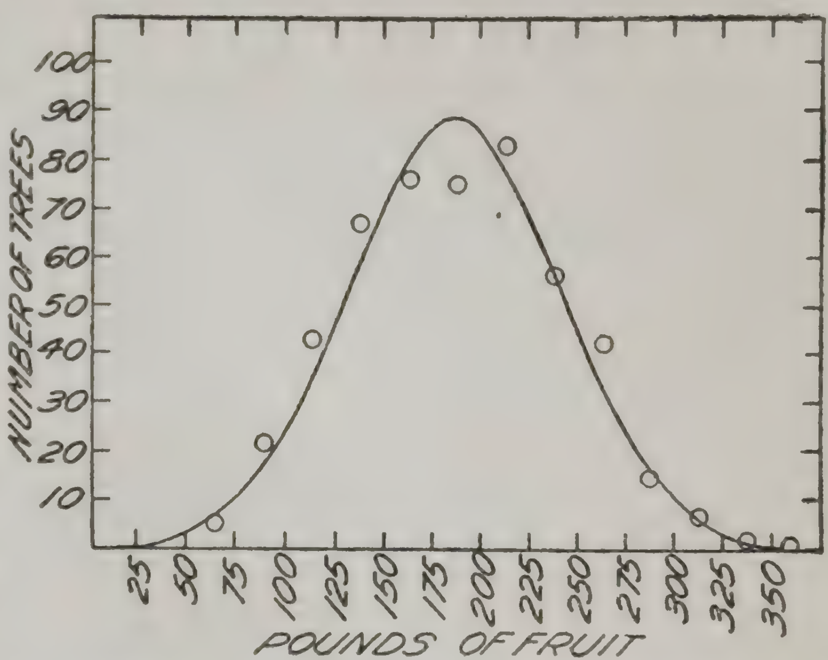

FIG. 11.—Curve of yield of individual trees, navel orange (Antelope Heights).

TABLE VIII.—*Comparison of the reliability of a plot of 16 adjacent trees with that of a plot of 16 trees of four scattered units of 4 trees each. Navel oranges (Antelope Heights)*

Plot.	Extreme yields per plot.	Mean yield per plot.	Standard deviation.	Probable error per plot (percentage of mean).	Difference necessary to give 10-to-1 assurance.	
					Percentage of the mean.	Observed yield.
	Pounds.	*Pounds.*	*Pounds.*			*Pounds.*
Adjacent trees	1,800 to 4,000	2,973±68	556±48	±12.61	±62.94	±1,871
Scattered unit plot	2,400 to 3,600	2,960±36	282±25	± 6.42	±32.05	± 949
Theoretical				± 5.01	±25.03	± 746

An example taken from the Jonathan apple orchard may well be considered. Suppose it is desired to know the necessary difference which must exist between plots of 16 trees each to give us the reliance of a 10-to-1 chance that it is due to differential treatment (a) when the plots are made up of adjacent trees, (b) when the plots are made up of two scattered units of eight trees each, and (c) when the plots are made up of four scattered units of four trees each.

The probable errors in the above cases, a, b, and c, are ±13.49, ±8.90, and ±5.69 per cent, respectively. By proceeding as before it is found that the necessary difference for a 10-to-1 chance is as follows: (a)

±67.35 per cent; (b) ±44.44 per cent; (c) ±28.42 per cent. Table IX summarizes the results. It seems probable, therefore, that a difference between two 16 adjacent tree plots of less than 50 per cent of the mean production should be considered with caution before attributing it to differential treatment. The scattering of the units of the plots increased the accuracy very decidedly, four units giving more accurate comparison than two. Even with this scattering, differences of less than 30 or 40 per cent are well within the realm of chance. The apparent cause for the 4-unit plot having a probable error less than the theoretical is accounted for by the fact that the variation in productivity of the soil was known when the distribution of the units was made. The results might not have approached so closely to the theoretical if the distribution had been decided upon before harvest.

TABLE IX.—*Comparison of the reliability of a plot of 16 adjacent trees with that of two units of 8 trees each and of four units of 4 trees each. Jonathan apples*

Plot.	Extreme yields per plot.	Mean yield per plot.	Standard deviation.	Probable error per plot (percentage of mean).	Difference necessary to give 10-to-1 assurance.	
					Percentage of the mean.	Observed yield.
	Pounds.	*Pounds.*	*Pounds.*			*Pounds.*
(*a*) 16 trees adjacent	3,000 to 6,500	4,864±176	974±124	±13.49	±67.35	±3,276
(*b*) 2 units of 8 trees	3,900 to 6,100	4,857±115	641± 82	± 8.90	±44.44	±2,158
(*c*) 4 units of 4 trees	3,800 to 5,400	4,871± 74	411± 52	± 5.69	±28.42	±1,384
Theoretical (based on random sampling)				± 6.95	±34.70	±1,687

The large probable errors which are apparently always present with plot trials of fruit trees emphasize the importance and value of obtaining individual tree records of experimental orchards before differential treatment is started. The probable errors will likely differ somewhat from year to year, and possibly be further influenced by the advanced age of the trees. Nevertheless, if but one or two years' records of mature trees are available before differential treatment is commenced, at least some idea can be obtained of the casual variation of the plots—that is, limitations can be placed beyond which observed differences in plots may be due to chance rather than to the factors under experimentation. In the absence of such previous records, the employment of frequent controls or standard treatments may be indicative of the probable error of the entire area.

RELATION OF THE SHAPE OF THE PLOT TO THE VARIABILITY OF THE COMPARATIVE YIELDS

The shape of small plots may be of great importance when cultural operations are considered.

Lyon (*1912*) found no satisfactory evidence that long and narrow plots are less likely to error than square plots when no control plots are

used, but when controls are placed every second or third plot in the series, the evidence is in favor of the long and narrow plots. The use of every second or third plot in the series as a control plot obtains greater accuracy than when no controls are used and the average of the field is considered the normal yield for all plots.

A long plot is much more economical of time and labor than a square plot containing the same number of trees. If the orchard must be irrigated, a square plot containing nine trees will require three standpipes instead of one and also three times as many irrigation furrows.

Arguments have been advanced from time to time in favor of both the linear and the square plot. The advocates of the former have urged its reliability on two points: first, if the soil or other conditions change in a direction parallel to the plot, it will contain both high- and low-yielding areas, and the average will correct one or the other error; second, if conditions change in a direction more or less perpendicular to the plots, each plot will vary from its adjoining plot because of its shape by such a minimum that intercomparisons are more reliable. On the other hand, the advocates of the square plot have claimed that the arguments apply equally well to their case, provided the plot be made small enough.

In view of the importance of the shape of the plot for cultural operations, the writers have investigated the variability of plots of various sizes and shapes in three of the experimental orchards. The results (Table X) are for unrepeated plots and for one year's yield of fruit, except the apples, which are for two years. Considerable interest lies in the computation on the 9-tree plot in the total navel-orange grove, because it is based on a large population (1,000 trees) and because the soil is known to vary in a general way from northeast to southwest.

Comparisons were made between square plots containing 9 trees and linear plots consisting of a single row of 9 trees extending in the north-south and in the east-west direction. It is interesting to see how closely the coefficients of variability coincide in the three cases. The differences between the coefficients are only a fraction of the probable errors. Therefore on this lot of 1,000 orange trees there is no difference between a square and a linear plot of 9 trees, so far as the reliability of comparative yields is concerned. (The deviations were taken from the mean of all the plots of the grove.)

Similar comparisons were made on a selected block of 256 trees in this same grove. The coefficient of variability of the trees taken singly is 34.47 ± 1.14, which indicates that these trees were a fairly typical sample of the entire grove. The block was divided into plots of various shapes containing 16 trees. With one exception there is little difference in the variability of the plots, regardless of their shape. This exception is found in the linear plot of 1 by 16 trees extending from east to west, which has a lower coefficient than any other arrangement. In the block

of trees chosen there is a gradual variation of soil in this direction, and these plots therefore include both high- and low-yielding trees in about equal proportions, the difference from plot to plot being small, as shown in Table X.

TABLE X.—*Effect on variability of changing the shape of plots*

Kind of trees.	Number of trees per plot.	Shape of plots.	Coefficient of variability.
Total navel-orange grove (Arlington)	9	3 by 3	28.18±1.48
Do	9	1 by 9 N.-S.	28.92±1.46
Do	9	1 by 9 E.-W.	28.36±1.43
Portion of navel-orange grove (Arlington), 256 trees	1	1 by 1	34.47±1.14
Do	16	4 by 4	16.77±2.05
Do	16	2 by 8 N.-S.	17.17±2.10
Do	16	2 by 8 E.-W.	15.39±1.88
Do	16	1 by 16 N.-S.	16.36±2.00
Do	16	1 by 16 E.-W.	10.09±1.31
Walnuts, 1915	4	2 by 2	31.09±1.93
Do	4	1 by 4	30.40±1.89
Do	9	3 by 3	23.94±2.32
Do	9	1 by 9 N.-S.	26.23±2.57
Do	9	1 by 9 E.-W.	22.46±2.16
Do	24	4 by 6	21.62±3.25
Do	24	1 by 24	6.56±1.00
Apples, 1914–15	8	2 by 4	19.32±1.81
Do	8	1 by 8 N.-S.	18.70±1.74
Do	8	1 by 8 E.-W.	21.35±2.18
Do	16	4 by 4	15.13±1.97
Do	16	2 by 8 N.-S.	16.41±2.15
Do	24	4 by 6	13.75±2.36
Do	24	1 by 24	12.81±2.20

Changing the shape of plots of walnut trees had little effect upon the coefficient of variability except in the case of the larger plots. The coefficients vary only slightly from one another, regardless of shape, in the case of the 4- and 9-tree plots. In the 24-tree plot, however, there is a great difference between the 4- by 6- tree plot and the 1- by 24- tree plot in favor of the latter. It should be borne in mind, nevertheless, that a plot of 24 walnut trees is an abnormally large plot, and in the example just referred to it is a plot 250 feet by 350 feet, compared with one 100 feet by 1,250 feet. This may take into consideration marked variation in productivity, even on apparently uniform soil.

In the case of the apple trees there is little difference between the variability of plots of equal size but different shapes, even in the case of the 24-tree plots. Regarding the question of the shape of the plot, it therefore appears that in most cases there is no difference in the variability of a linear or a square plot.

proportional to their distance from the plot. This would obviously be

In the case of a large number of trees, where a linear plot is long enough to include both high- and low-yielding areas in each plot, there appears to be an advantage in favor of the linear plot. The great advantage of the linear plot in most cases is the economy of cultural operations. Another advantage will be discussed in the following section on the interpolation of control plots.

A carefully conducted fertilizer experiment with orchard trees requires a guard row between adjacent plots in order to eliminate the possibility of the fertilizer's affecting the margin of the next plot, since there are many observations which show that tree roots extend considerable distances and often reach the lines of adjoining rows (*Hedrick, 1914; Ballantyne, 1916*). It is obvious that it is more reliable to employ guard rows rather than to divide cultural treatments midway between tree rows. The number of guard trees required for square plots is smaller than for a linear plot containing the same number of trees. A 9-tree plot in the form of a square requires 7 guard trees; in the form of a linear plot it requires 11 guard trees. In the former case seven-sixteenths of all trees are in the guard rows, in the latter eleven-twentieths. As the size of the plot increases, the difference becomes greater. It thus becomes a question of the extent to which one is willing to go in enlarging the size of the linear plot before the increase in the number of trees in guard rows offsets the economy in cultural operations thus obtained.

USE OF CONTROL PLOTS

INTERPOLATED CONTROL PLOT

Agronomists are in the habit of using every third, fourth, or fifth row or plot in the experimental tract as a standard from which the normal yield of any intervening treated plot may be calculated. The nature of the crops and the cultural methods used commend this system of arrangement.

This method, like others, has its advantages and disadvantages. After the results are obtained there is still a need for a proper method of comparison. There are several different methods of estimating the "normal" yield of any plot. The "normal" (N) may be estimated by the formalu

$$\frac{C_1+C_2+\ldots C_n}{n}$$

which is simply the mean of all the control plots in the area. If the soil of the area were uniform and all variations in the yield of the controls were purely chance variations, this method would give a satisfactory result. Again N may be estimated from the yields of the two nearest control plots. For example, if every third plot is a control and the arrangement is C_1, A, B, C_2, and so on, the normal for A would be $\frac{2}{3}C_1+\frac{1}{3}C_2$. In this way the yields of the controls receive weights inversely proportional to their distance from the plot. This would ob-

viously be satisfactory, provided there was no difference in the amount of variation between the plants on different plots and that the soil varied uniformly in one direction.

The first method mentioned computes the "normal" (N) for the whole area; the second, for a locus on that area. These normals may be combined to represent the resultant of both general and local conditions. Thus, the formula $N = \frac{1}{2}\ (C + \frac{2}{3}C_1 + \frac{1}{3}C_2)$ indicates that N is the mean of the values for N computed by the two preceding formulas. This assumes an equal value for the adjacent control plots and the mean of all control plots. Its use as N brings up the calculated yields of plots on low-yielding areas and reduces the same on high-yielding areas. In the case of cereals there is usually small chance for difference in the yielding powers of a plot and its nearest control on account of their proximity; but in the case of orchard trees situated some distance apart there may be greater soil changes between adjacent plots, and consequently a marked difference in yield, aside from the effect of treatment, between a plot and its nearest control plots. The introduction of the mean of all control plots might be expected to introduce a stabilizing factor. In the formula $\frac{1}{2}\ (C + \frac{2}{3}C_1 + \frac{1}{3}C_2)$ the mean of all control plots has equal weight with the normal derived from the nearest controls. Since the soil over any but very small areas may not be uniformly variable, it might seem more logical to weight the normal derived from the nearest controls more heavily than that derived from the mean of all control plots, and to combine the two. This has been done by Olmstead (*1914*) and others, making the formula $\frac{p_1C + p_2(\frac{2}{3}C_1 + \frac{1}{3}C_2)}{p_1 + p_2}$ in which p_1 and p_2 are constants arbitrarily chosen. Stockberger (*1916*) found satisfactory results by assigning the values $p_1 = 1$ and $p_2 = 3$.

The method used by the Office of Cereal Investigations, of the Bureau of Plant Industry, is $\frac{1}{2}(c + C_1)$, which employs half the sum of the mean of all control plots and the yield of the nearest control plot as the normal for any given plot.

Stockberger (*1916*) compared the relative precision of these formulas in computing the normal yields of plots of hops. Using the yields of six years he obtained the greatest precision from the formula

$$\frac{p_1C + p_2(\frac{2}{3}C_1 + \frac{1}{3}C_2)}{p_1 + p_2}$$

though no formula maintained the same relative rank throughout the six years. It would appear that there is no way of determining in advance the formula best suited to any particular case, at least not until more applications of the different formulas have been studied.

The five formulas above stated have been tested on the Arlington grove of navel oranges. The grove was parceled into linear plots of 10 trees each. Each alternate plot was designated as a guard row and

discarded from the calculation. This left 32 "treated" plots whose "normal" yields were computed. The arrangement of these plots and their yields are given in Table XI.

TABLE XI.—*Arrangement and yields of 10-tree plots of the navel-orange grove (Arlington)*

[Yield expressed in tens of pounds]

80 control	116*	108G	84*
105*	152 control	150*	88B
88H	184*	112 control	119*
115*	155A	124*	146 control
117G	164*	121F	151*
94*	154B	159*	174C
109 control	160*	142E	132*
118*	163 control	148*	182D
94F	147*	139 control	166*
76*	122C	153*	174 control
83E	142*	177D	180*
97*	137D	179*	169E
104 control	111*	176C	131*
101*	131 control	188*	162F
99D	148*	183 control	183*
117*	154E	178*	147 control
100C	165*	203B	188*
112*	134F	182*	168G
74 control	111*	206A	223*
101*	114 control	199*	201H
112B	109*	219 control	191*
107*	123G	191*	228 control
98A	78*		
108*	97H		
80 control	80*		
108*	106 control		
108H	137*		
110*	135A		

Computing the normal yields of these plots by the five formulas above described gives the results shown in Table XII.

TABLE XII.—*Value of different formulas for computing the coefficient of variability and probable error of yields of oranges in 10-tree plots in Arlington grove*

Formula used.	Standard deviation.	Coefficient of variability.	Probable error (percentage of mean).
	Pounds.		
1. $\frac{C_1+C_2 \ldots\ldots +C_n}{n}$	363±31	26.55±2.39	17.91
2. $\frac{2}{3}C_1+\frac{1}{3}C_2$	171±14	12.52±1.07	8.44
3. $\frac{1}{2}(C+\frac{2}{3}C_1+\frac{1}{3}C_2)$	219±18	16.01±1.38	10.80
4. $\frac{p_1C+p_2(\frac{2}{3}C_1+\frac{1}{3}C_2)}{p_1+p_2}$	180±15	12.93±1.11	9.00
5. $\frac{1}{2}(C+C_1)$	233±20	16.78±1.46	11.32

* Guard row.

These figures show that there is less error in this case by the use of formulas (2) and (4) and that there was very little difference between these two.

This question of a "normal" yield of a plot depends obviously upon the portion of the population chosen as a standard and upon the method of calculation. Investigators differ in the choice of both of these factors.

An illustration may be given to show the results of calculating the normal from different standards. The coefficient of variability of the 10-tree plot of Arlington navel oranges was computed, taking the deviations from (a) the mean of all plots, (b) the mean of all control plots, and (c) the "normal" calculated by formula 2. The results are shown in Table XIII.

TABLE XIII.—*Deviation of yields from mean of area compared with deviation from 10-tree linear control plot*

Formula No.	Deviation.	Coefficient of variability.
1	Deviation taken from mean of all plots	26.00 ± 2.30
2	Deviation taken from mean of control plots	26.55 ± 2.39
3	Deviation taken from normal calculated from nearest control $\frac{2}{3}C_1+\frac{1}{3}C_2$	12.5 ± 1.10

Since no differential treatments had been given these plots, the mean of all plots differs little from the mean of all control plots, the respective means being 1,376 and 1,367 pounds. The variability of plots calculated from these two standards is not significantly different. In the case of the deviation taken from the normal yield, however, there is a very significant decrease in variability, since there are discontinuous soil variations not recognizable in the general average of the area which are taken into account by this formula.

CHANGE ARRANGEMENT OF CONTROL PLOTS

A weakness of this system of comparison with interpolated plots lies in the possibility that the series of control plots may not be representative of the area. The plots chosen for controls may be on soil superior or inferior to that of the intervening plots. There are indications that this possibility may be more real than one would expect from purely random sampling.

A few computations will show the extent to which the different methods of choosing control plots may affect the results. The Arlington grove records were recomputed, shifting the control plots back one row. The yield of the first control plot (see Table XI) was 1,051 pounds instead of 800, and the last was 1,160 instead of 1,520 pounds. The first arrangement will be termed "arrangement A," the second "arrangement B." The mean yield of all control plots is not greatly changed by the different

arrangement. The mean of the "A" control plots is 1,367 pounds, that of the "B" control plots is 1,389 pounds, yet, as shown in Table XIV, the coefficient of variability is increased from 12.5 ± 1.1 in "arrangement A" to 18.2 ± 1.6 in "arrangement B" for single plots, with similar increases for the repeated plots.

TABLE XIV.—*Effect of two methods of arranging control plots*

Arrangement of plots of 10 trees.	Coefficient of variability.			
	Repeated once.	Repeated twice.	Repeated three times.	Repeated four times.
Arrangement A (Arlington)...	12.52 ± 1.07	7.53 ± 0.89	7.68 ± 1.17	4.33 ± 0.73
Arrangement B (Arlington)...	18.25 ± 1.59	16.07 ± 1.97	12.46 ± 1.91	8.75 ± 1.50

The records of Stockberger's hop yields have been computed in the same manner. The coefficient of variability of the plot yields in his arrangement is 16.71 ± 3.86. Moving the control plots down one—that is, using plot *A* as c_1 and so on—produces a coefficient of variability of 15.93 ± 3.55. There is obviously no difference between these two values.

The problem was also investigated by the use of the 8-tree plot lemon records. In this case three possibilities were tried, with the first, second, and third plots in turn as c_1 and every subsequent third plot as a control plot. The coefficients of variability for the different arrangements were 21.7 ± 2.21, 22.5 ± 2.29, and 23.8 ± 2.82. In view of the probable errors of the coefficients, there seems to be no real difference in the result of the different arrangements in this grove.

Since a decided difference was found in one case out of the three studied, it would seem that there is a rather high probability that significant differences may result from different arrangements of control plots.

VARIABILITY IN THE YIELDS OF MORE THAN ONE YEAR

It is often assumed that the mean yield of two or more years is less variable than the yield of one year.

We have had opportunity to study the variability in the yield of 60 navel-orange trees over a period of several years. The data were kindly furnished us by Mr. A. D. Shamel, of the Bureau of Plant Industry. The trees in question had been selected for their uniformly good production and the individual yields recorded for six years.

The figures presented in Table XV show that the variability of yields fell off distinctly after one year, but the reduction was negligible after the yields of two years were combined. It will be noted that the coefficient of variability of the single trees even for one year is notably low. Considering the records of single trees, the average of six years' records is not less variable than the average of two.

TABLE XV.—*Comparative variability of yields of navel-orange trees through a period of six years*

Class.	Coefficient of variability of the yield of—					
	1 year.	2 years.	3 years.	4 years.	5 years.	6 years.
Individual trees	22.8±1.5	15.0±0.9	14.7±0.9	13.3±0.8	13.7±0.9	14.6±1.0
Plots of 10 trees each	3.9±0.8	2.9±0.6	2.7±0.5	2.6±0.5	3.4±0.7	3.9±0.8

The coefficient of variability for the 10-tree plot is remarkably low for the reason that the trees themselves are so uniform and only a small area of ground is involved. It is no surprise, therefore, to find that the coefficients of variability are nearly equal, calculated from one to six years. The probable errors are relatively large and it is difficult to assert that there is any real difference.

The walnut yields can be used as additional data for the study of this question. Table XVI shows the coefficient of variability of the 1915 and 1916 yields and their total, with both 1- and 8-tree units. By considering the individual tree as a unit, the total yield for the two years was less variable than the 1916 yield, the difference equaling three times the probable error. The variability of the 1915 yield is practically equal to that of the total. On considering an 8-tree plot as a unit, there is a difference between the coefficients for 1915, 1916, and the total of the two years respectively; however, the observed difference is less than three times the probable error and its significance may be somewhat doubted. Apparently the mean of two years' yields in this case is less variable than one year's yield.

TABLE XVI.—*Comparative variability of yields of seedling walnut trees through a period of two years*

Class.	Coefficient of variability of the yield.		
	1915 yield.	1916 yield.	Mean of 1915 and 1916 yields.
Individual trees	47.9±1.6	53.9±1.9	46.4±1.6
Plots of eight trees each	30.0±2.6	33.3±3.0	25.3±2.2

Further studies on the comparison of the variability of yields through several years were made, from data published by Hedrick (*1911*). Table IV of the bulletin cited gives the yearly yields of individual apple trees from 1902 to 1910, inclusive, upon which our computations are based.

Four differential fertilizer treatments have been given to eight plots of five trees each, each treatment being duplicated on nonadjacent plots. There are three nontreated plots serving as controls.

The variability of the individual trees was computed on the 15 control trees.

The variability of plots is based on hypothetical plots made up of one tree from each of the treated and one from each of the untreated plots. These hypothetical plots are therefore made up of an equal number of trees having similar treatments. The variability of the 10 plots thus obtained was computed for the single year 1910, and for the sum of two, three, four, and seven years. Table XVII gives the coefficients of variability for single trees and for 5-tree plots.

TABLE XVII.—*Comparative variability of yields of Baldwin apple trees through a period of seven years* [a]

Class.	Coefficient of variability of the yield.				
	1 year (1910).	2 years (1909 and 1910).	3 years (1908–1910).	4 years (1906, 1908–1910).	7 years (1902, 1903, 1905, 1906, 1908–1910).
Individual trees..	37.3±5.2	33.6±4.6	32.5±4.4	32.2±4.4	34.0±4.7
Plots of five trees each...........	18.5±2.9	16.8±2.6	18.1±2.8	18.2±2.8	21.7±3.4

[a] Records taken from Hedrick (*1911, p. 172–174*).

It is interesting to note how slightly the variability of the yields is decreased by combining two or more years. If regard is paid to the probable errors, it can not be said that there is any real difference. In other words, one year's records of the yields of these apple trees seem to be as reliable for variation studies as those for several years.

It seems, therefore, that the continuation through several seasons may not so materially decrease the variability of tree yields as one might expect. This has a direct bearing on the reliability of the major portion of the calculations of this paper which are based on the variability of the yields of one season. It should be kept in mind, however, that these studies do not concern the relative yield of one plot compared with another, but rather deal with the total variation from the mean of the yields of all the plots. As cited before from the work of several experimenters, the relative productivity of a group of plots may not be fully determined even after a period of years, whereas the tree yields from Hedrick, Shamel, and the data of the writers indicate that a measure of the variability for one year of a group of trees divided into plots, may be very representative of the mean variability for several years.

SUMMARY

(1) The present paper is the result of a study of the nature and extent of the casual variability of yields of fruit trees under field conditions and its bearing on the reliability of plot trials.

(2) Studies have been made upon the variability of the yields of orange, lemon, apple, and walnut trees. The orchards studied were selected on

account of uniformity of treatment and appearance, yet the variability in productivity was considerable. The coefficient of variability for the yield of individual trees of the clonal varieties ranged from 29.27 ± 0.69 to 41.23 ± 1.52 per cent, but for the individual seedling walnuts, the coefficient was somewhat higher, reaching 53.91 ± 1.92 per cent. The variability of these tree yields approaches the normal curve of errors. This variability may be assumed to be the result of "casual" factors which are beyond the control and possibly the recognition of a careful experimenter.

(3) The effect upon variability of combining trees into plots of various sizes and shapes has been investigated. As the number of trees per plot is increased, the coefficient of variability decreases. The coefficient of variability does not decrease, however, in proportion to the increased number of trees per plot. In most cases there is little gained in accuracy by increasing the plot to include more than eight adjacent trees.

(4) One of the great causes of variability in yields appears to be the heterogeneity of apparently uniform soil. While a combination of a sufficient number of adjacent trees into a plot will overcome largely the fluctuations of individuals, nevertheless the plots may not sufficiently include both high- and low-yielding areas to give a typical average. Greater reliability may be secured by a systematic repetition and distribution of plots through the experimental area. A consistent gain in reliability resulting from this method of repetition is shown by the use of several different methods of computing the variability.

The coefficient of variability for an average plot of 16 adjacent trees was 22.58 ± 1.01, while 16 trees in four scattered ultimate plots each of four trees have a coefficient of variability of 9.29 ± 0.40. The larger the number of units in a combination plot the more typical is the sample of the area obtained. A 16-tree plot can be expected to give more reliable results if divided into four equal plots and repeated at four regularly placed intervals than can either two 8-tree plots, or 6 adjacent trees. The same principle holds true for larger units. A given number of unit plots will give a greater accuracy than half the number of units with twice as many trees per unit.

Four repetitions of an ultimate plot reduced the coefficient of variability to a point considered practical for cultural operations. Further repetitions, though reducing the coefficient in less degree, did not appear to justify the additional number of trees required. A minimum of 8 to 10 trees is required for plots involving cultural experiments. In the case of rootstock, pruning, or variety trials, twice as many plots each containing half as many trees might be used to obtain greater accuracy.

The fact that marked soil variations occur which tend to make adjacent trees or adjacent plots yield alike, even on soils which were chosen because of their apparent uniformity, is well shown by applying the formula proposed by Harris (*1915*) for measuring the coefficient of correlation between neighboring plots of the field. Applying this to the Arlington

navel oranges, the writers have calculated the correlation between the yield of the 8-tree plot as the ultimate unit and the yield of the combination of four such adjacent plots, and it was found that

$$r = +0.533 \pm 0.085.$$

This result shows a marked correlation, indicating a pronounced heterogeneity in the soil of this grove influencing fruit production.

However, when the correlation between the 8-tree plot as the ultimate unit and the yield of the combination of four such systematically scattered plots was calculated it was found that

$$r = +0.137 \pm 0.120.$$

This coefficient is practically equal to its probable error and can be regarded as significantly zero.

(5) In the computations made by the writers emphasis is also laid upon the nature and magnitude of the probable error. It is shown in several cases that the probable error of comparison between plots may be so large that relatively large differences must be evident between treated and untreated plots for a reasonable assurance that it is due to the factors being experimented upon. With the plots of 16 to 32 adjacent trees which were studied, a difference of from 62.94 to 81.97 per cent of the mean production would be necessary in order to obtain chances of 10 to 1 that the results were due to differential treatment and not to casual variation in the productivity of the trees. With the same number of trees in scattered units, a difference of 28.42 to 50.02 per cent would be necessary for the same odds. It seems probable, therefore, that a difference between two tree plots of less than 50 per cent of the mean production should be considered with caution before attributing it to differential treatment.

(6) The relation between the shape of a plot and its variability was investigated by making comparisons between square plots and linear plots containing the same number of trees. Except in the case of large plots, the difference in the variability of plots of different shapes was insignificant.

(7) In any method of field experimentation where a standard of comparison is desired the theoretical or "normal" yield of a plot is a question of importance. By the use of certain formulas the "normal" yield may be computed from control plots. As a standard, one may use the average yields of the control plots of the entire area, or of the nearest control plots, or a combination of the two. In cases studied, the coefficient of variability was reduced 50 per cent by calculating the normal yield from the nearest controls in place of using the mean of the entire area. The employment of every alternate row as a control plot was not sufficient to offset the variability due to soil heterogeneity.

(8) Computations made on the yields of orange, walnut, and apple trees for several consecutive years showed little annual fluctuation in their variability. One or two crops may not show greater variability than the average of six or seven crops.

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INTERRELATIONS OF FRUIT-FLY PARASITES IN HAWAII

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INTRODUCTION

The introduction and ultimate establishment of four species of larval parasites of the Mediterranean fruit fly (*Ceratitis capitata* Wied.) in Hawaii, wherein exist ideal conditions for the rapid and unchecked development of the host throughout the year, has opened an exceptional opportunity for an investigation of all phases of the work of these parasites, not only in their relations to the host but also to one another, and has made possible the determination of many points of unusual interest to biological students of insect parasitism and of particular value to entomologists dealing with considerations relating to general parasitic control of insect pests.[1]

The information herein presented is given, not as a final decision or positive argument against the introduction of many parasites of a single pest, but to reveal the actual need for careful biological studies of parasites, especially in their interactions upon one another, before general ntroductions or liberations can be intelligently undertaken.

Entomologists, detailed in the past for researches in insect parasitism in foreign countries, have customarily adopted the policy of assembling all available species of primary parasites of the insects under investigation, with the final intention of conveying them all to the home country for propagation and liberation. The chief caution of these workers has usually been the elimination of all secondary parasites from the material prepared for shipment. Admirable results have often been achieved. However, after the introduction of several species, through one or successive importations, few, if any, considerations have been given to the possibility of detrimental results arising from interference with the action of one parasite of primary importance and great prolificness by another of less value and proved inferiority.

The important bearing that a preliminary and detailed knowledge of parasite habits may have upon the general question of parasite importations has already been well directed to the attention of entomologists by Dr. L. O. Howard. He states[2] that—

> It is unwise and most unpromising to attempt heterogeneous and miscellaneous importations of parasites without careful study of the host insect on its home ground

[1] For a history of these parasitic introductions and a discussion of climatic and host relationships favoring parasitic increase, see BACK, E. A., and PEMBERTON, C. E., THE MEDITERRANEAN FRUIT FLY IN HAWAII. U. S. Dept. Agr. Bull. 536, 119 p., 21 pl., 24 fig. 1918.

[2] HOWARD, L. O. THE PRACTICAL USE OF THE INSECT ENEMIES OF INJURIOUS INSECTS. *In* U. S. Dept. Agr. Yearbook 1916, p. 282. 1917.

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and in its natural environment throughout the whole range of its existence and a similar biological study of its parasites and natural enemies under such conditions.

Some results of the recent work in Hawaii most strongly bear this out. Sufficient evidence has been obtained to throw serious doubt upon the assumption, often accepted, that the greater the number of species of parasites associated with the host, the greater the chances for its control. It is felt that the following data quite definitely indicate, at least in some cases, that better results may be obtained by a method of judicious selection of desirable species for introduction rather than by a wholesale and indiscriminate procedure. If 90 per cent of all individuals of an insect pest are destroyed by a single species of parasite, is it wise to attempt further control by bringing in other species, until it is known by positive and careful experimentation in the laboratory and field that these new species will not interfere with or check the normal activities of the first species?

In May, 1913, Prof. F. Silvestri succeeded in bringing two species of opiine parasites of the fruit fly into Hawaii. One, *Opius humilis* Silvestri, he brought from South Africa, and the other, *Diachasma tryoni* Cameron, was secured in Australia. Both were soon established in the Kona coffee district of the island of Hawaii. By 1915 it had become clearly evident that *O. humilis* was often parasitizing from 60 to 90 per cent of all of the fruit-fly larvæ developing in the coffee cherries. *D. tryoni* steadily but slowly increased and in time exhibited a capacity for occasionally parasitizing 50 per cent or more of the host larvæ. Here it is obvious that overlapping or duplication in parasitism was occurring. Clearly some fly larvæ were being stung by both species of parasites and frequently to a very considerable extent.

CANNIBALISM AMONG THE PARASITES

Early in 1916, Dr. E. A. Back, of the Bureau of Entomology, while examining the contents of some parasitized fruit-fly material from the field, observed under the microscope a larva of the parasite *Diachasma tryoni* attacking one of the parasite *Opius humilis*. A suspicion of disadvantageous consequences arising from complications attending the interactions of these parasites led Mr. C. L. Marlatt to assign to the writers an investigation of this subject.

Careful microscopical examinations of large numbers of fruit-fly larvæ and pupæ, collected from localities where both species of parasites were known to be well established and actively working together, soon revealed one striking fact. In the majority of cases where fruit-fly larvæ had been parasitized by both *Diachasma tryoni* and *Opius humilis* the latter was killed and the former developed to maturity. *O. humilis* is killed purely by wounds and lacerations inflicted upon it by the long, curved, sickle-like mandibles of the newly hatched larva of *D. tryoni*. This larva

attacks the young of *O. humilis*, usually at a point a few segments back of the head, the point of contact being clearly seen in Plate 12. Its mandibles open wide and snap into the body of the attacked larva spasmodically and with remarkable quickness. Often the entire operation of broadly opening and closing the mandibles may be almost imperceptible to the eye. It may move its entire body quickly, the caudal tip may be curled beneath the body and extended again suddenly, and the mandibles may be repeatedly opened and closed until successfully grasping the Opius larva. Besides possessing unusual powers for inflicting injury to other parasitic larvæ about it, it may avoid counterattack through ability to move quickly and through the protection afforded the entire ventral surface of the body by a thick mass of serosal, cellular material that accompanies the larva when it emerges from the egg, and which remains with it during its entire life in the first instar. This mass of cells may be seen clinging to Diachasma larvæ of the first instar in Plate 10.

The newly hatched larva of *Opius humilis* possesses mandibles which are also long and pointed, as shown in Plate 12, A, B. These may be used to good advantage when the larva is successful in bringing them in contact with individuals of its own or of other species of parasites. The larva, however, is sluggish, moves much less quickly than that of *Diachasma tryoni*, is protected ventrally by a much thinner, less adhesive mass of serosal cells, is much less capable of quick and powerful movement of the mandibles, and usually holds the body in a somewhat horizontal and exposed position. These deficiencies seem to explain its inability to avoid destruction by larvæ of *D. tryoni* or to offer successful counterattack when the two are lodged within the same host larva.

During the examination of nearly 3,000 fruit-fly larvæ or pupæ, parasitized in each case by the two species of opiines, the dead or dying and often struggling Opius larvæ were frequently dissected from the tissues of the host while still tightly clasped in the mandibles of the Diachasma larvæ. Plate 10, A, is reproduced from a photomicrograph of a larva of *O. humilis* actually within the grasp of an active, living larva of *D. tryoni*. In this particular case the operation of removing the two larvæ from the host, placing them upon a microscope slide in water, and covering them with a cover glass failed to separate them, and the Diachasma larva ultimately died with its mandibles deeply embedded in the body of the dead Opius larva in the exact position as shown. Plates 12 and 13, A, B, C, show larvæ of *O. humilis* in various stages of laceration and distortion just as they were removed from fruit-fly larvæ or pupæ in which were also one or more larvæ of *D. tryoni*.

Extensive laboratory experiments have exactly confirmed the results of the first series of field observations. The aggressive, cannibalistic period of activity of the larvæ of these parasites is during existence in

the first instar, and particularly during the early period of this stage, before the body becomes engorged and swollen with food. Plate 11, B, which shows a mature first-instar larva of the parasite *Diachasma tryoni*, is interesting in this connection when compared with the other illustrations of newly hatched Diachasma larvæ of this instar. The head is unchanged in size, but the body is greatly distended after two days of feeding preparatory to molting to the second instar. In all of the illustrations the enlargement is the same. It is obvious that the enlarged and somewhat rigid body of the well-fed larva permits much less freedom of movement than is possible shortly after hatching. The body is first elastic, flexible, and capable of quick and effective action. This change in the size of the body may occur in from two to two and one-half days.

The molt to the second instar still further incapacitates the opiine larva for carrying on further cannibalistic action. The mandibles are then small, soft, and are almost imperceptible, even under high magnification, because of their transparency, being wholly unfitted for active use except in the separation of the semiliquid media in which the larva lies after the host has formed the puparium. The strong and heavily chitinized head of the opiine larva in the first instar is thus entirely discarded upon the molt to the second instar, and all further cannibalism ceases. The helpless condition after the first molt is well suggested in Plate 11, A, which shows a larva of *Diachasma tryoni* in the second instar.

During 1916 a microscopical examination of the contents of a total of 2,925 parasitized fruit-fly larvæ and pupæ definitely showed that the parasites readily oviposit in the same host larva more than once and exhibit no discernible instinct of selection of parasitized or unparasitized larvæ. All eggs so deposited hatch. Thus, fly larvæ commonly opened were found to contain several eggs or larvæ of the two species of parasites and sometimes a third species (*Diachasma fullawayi*), a later introduction.

Here was certain evidence of a complicated overlapping or duplication of parasitism. In no single instance were two parasite larvæ ever observed to develop to maturity in the same host larva, except in the case of the chalcid *Tetrastichus giffardianus* (p. 292). All but one were killed while still in the first instar, or occasionally before hatching. At times from 8 to 10 opiine larvæ were found within the host larva. Only one would mature and, as a rule, if a larva of Diachasma were one of the number, it survived all others.

If several eggs of *Diachasma tryoni* or *D. fullawayi* alone are deposited in an individual host larva over a period of two or three days, the last parasite larva to hatch stands the best chance for destroying the others and maturing. The case is identical with the *Opius humilis* However, a well-grown and fully-fed larva of *D. tryoni* or *D. fullawayi*, if still in the

first instar, seems entirely able to destroy larvæ of *O. humilis* that are newly hatched and unencumbered with a body engorged with food.

All opiine larvæ within an individual fruit-fly larva or pupa may sometimes be fatally wounded and no parasite develop. This is not frequent. Often the deposition of 8 or 10 parasite eggs into a single fly larva causes its death. In such cases the parasite eggs usually hatch and the resulting larvæ die within a short time.

Cases occurred in which as many as 10 dead larvæ of *Opius humilis* were dissected from a fruit-fly larva, together with a single, vigorous, active larva of *Diachasma tryoni*. This does not necessarily mean that all of the 10 larvæ were killed by the latter. No doubt some of the former larvæ destroyed each other, but it clearly shows the superior aggressive and defensive power of the larva of *D. tryoni*. Many cases have been observed in which a larva of *O. humilis* was badly cut and distorted from attack by a larva of *D. tryoni*. Some such cases are shown in Plates 10, 12, and 13. Occasionally the body may be found entirely severed from the head. These extreme cases are no doubt caused by reattack upon the larva a day or more after it has died and has become somewhat softened. In most cases the death of the larva seems to be caused by the first grasp or pinch of the attacking larva. A single perforation in the body wall should be sufficient to cause the death of the larva in a short time.

Occasionally an opiine larva will destroy eggs of its own kind when it occurs in the same individual host with the eggs. In this manner mature embryos are sometimes very much distorted and almost unrecognizable. This is not frequently seen.

Cool weather materially retards the development of the opiine egg, particularly in the case of *Diachasma tryoni* and *D. fullawayi*. At such times many cases have been observed in which larvæ of *Opius humilis* have developed to the second instar before an egg of a species of Diachasma, which had been deposited into the same host larva harboring *O. humilis*, had hatched. Upon hatching the small, active Diachasma larva quickly destroyed the large, bulky Opius larva. This unusual condition has been observed only in January in the cool, elevated, coffee districts on the Island of Hawaii.

From over 2,900 cases where parasitized fruit-fly puparia have been opened, in no single instance has a case been observed in which the host pupa was formed. A host larva once parasitized quite readily forms into a normal puparium when sufficiently developed, but the presence of a single small opiine egg within its body invariably prevents any further development. The puparium is formed, the histolysis of the tissues is completed, and here all development of the fruit fly ceases. The broken-down and liquid medium thus prepared within the puparium, in which the parasite larvæ may move about and feed, enables them to reach all portions within and easily to come in contact with any other parasitic individuals that may occur there with them.

Thus, the inevitable tendency of every individual opiine larva upon hatching is to destroy every other parasitic larva in its domain, whether it be one of its own kind or of another species. This would appear to be an infallible instinct and one of great consequence in Hawaii in the development of the opiine parasites now present.

SUPPRESSION OF OPIUS HUMILIS BY DIACHASMA TRYONI AND D. FULLAWAYI

The parasites *Diachasma tryoni* and the closely related *Diachasma fullawayi*, by virtue of this larval instinct, coupled with further endowed superior body characters, have been responsible for the great suppression of the parasite *Opius humilis*. The last-named species is more prolific and hardy than either of the two others and is more generally efficient than both combined. By their association with the *O. humilis*, they have worked a detriment by reducing the total extent of parasitism to a point below that to which it is capable of exerting alone. The evidence of such suppression, gained from microscopical examinations of fruit-fly larvæ and pupæ secured from various fruits in Hawaii during 1916 and 1917, may be expressed as follows:

From April 16 to May 10, 1916, a dissection was made of 757 fruit-fly pupæ, freshly secured from coffee collected in the Kona coffee district of the Island of Hawaii. From this total, 345 were parasitized by only *Opius humilis*, 90 contained living larvæ of *Diachasma tryoni* together with dead larvæ of *O. humilis*, 9 contained living larvæ of *O. humilis* together with dead larvæ of *D. tryoni*, 1 was parasitized by only *D. fullawayi*, 5 contained living larvæ of *D. fullawayi* together with dead larvæ of *O. humilis*, 2 contained living Opius larvæ together with dead larvæ of *D. fullawayi*, 57 contained living larvæ of only *D. tryoni*, and 248 were not parasitized. Here it is seen that from the total of 757 puparia, 106 cases of duplication in parasitism occurred in which a species of Diachasma was found in the same puparium with one or more individual larvæ of *O. humilis*, and that in 95 of these the latter was killed and the former survived.

A further series of microscopical examinations of the contents of fruit-fly puparia, freshly secured from coffee fruits in this same district in January, 1917, during the coolest part of the year, strongly confirmed the results of the previous year. Six hundred and twenty-seven puparia were opened and examined. Of these a total of 343 were parasitized by *Opius humilis* alone, 67 were parasitized by only *Diachasma tryoni*, 2 contained only larvæ of *Diachasma fullawayi*, 129 contained living larvæ of *D. tryoni* together with dead larvæ of *O. humilis*, 8 contained dead larvæ of *D. tryoni* together with living larvæ of *O. humilis*, 4 contained living larvæ of *D. fullawayi* together with dead larvæ of *O. humilis*, 2 contained dead larvæ of *D. fullawayi* together with living larvæ of *O. humilis*, and 72 puparia were unparasitized. These results are again very significant.

From the total of 627 puparia 143 cases are noted in which an overlapping in parasitism occurred, wherein the puparia in each case contained larvæ of *O. humilis* in combination with larvæ of a species of Diachasma, and in 133 of these cases the former was destroyed.

The collection of extensive data on the percentage of parasitism exerted by the fruit-fly parasites in Hawaii over a period of three years gave abundant proof that the parasite *Diachasma tryoni* was most active during the warmer months of the year. This increase in activity and abundance paralleled a reciprocal decrease in the abundance of *Opius humilis*. The reverse was true during the remainder of the year, when the former species rapidly decreased and the latter ascended to a place of first importance. The data presented in Table I most positively reveal the extent of fluctuation in the comparative abundance of *O. humilis* and *D. tryoni*, the effectiveness of *O. humilis* being clearly at its maximum during the spring, when the abundance of *D. tryoni* is at its lowest, owing to the accumulated effect of the cool winter months.

TABLE I.—*Comparison of seasonal abundance of Opius humilis and Diachasma tryoni*

Locality.	Date of collection of host.[a]	Number of *Diachasma tryoni* emerging.	Number of *Opius humilis* emerging.	Percentage of *Diachasma tryoni.*	Percentage of *Opius humilis.*
Honolulu, Oahu	Mar., 1916	30	1,000	2.9	97.1
Do	Apr., 1916	1,200	9,778	10.9	89.1
Do	May, 1916	499	2,127	19.0	81.0
Do	June, 1916	2,303	998	69.8	30.2
Do	July, 1916	1,786	549	76.5	23.5
Do	Aug., 1916	2,286	649	77.9	22.1
Do	Sept., 1916	4,514	1,139	79.9	20.1
Do	Oct., 1916	6,772	2,061	76.7	23.3
Do	Nov., 1916	4,451	1,206	78.9	21.1
Do	Dec., 1916	2,605	1,602	61.9	38.1
Do	Jan., 1917	1,406	679	67.4	32.6
Do	Feb., 1917	558	1,101	33.6	66.4
Kona District, Hawaii	Sept., 1915	330	118	73.6	26.4
Do	Dec., 1915	210	274	43.3	56.7
Do	Mar., 1916	85	440	16.1	83.9
Do	Apr., 1916	756	3,031	19.9	80.1
Do	Aug., 1916	271	77	77.9	22.1
Do	Jan., 1917	558	4,749	10.5	89.5

[a] For similar data for 1915, see BACK, E. A., and PEMBERTON, C. E., THE MEDITERRANEAN FRUIT FLY IN HAWAII. U. S. Dept. Agr. Bul. 536, 119 p., 21 pl., 24 fig. 1918.

From September to December, 1916, a microscopical examination was made of the contents of 618 fruit-fly puparia obtained about Honolulu from coffee, guavas, and the winged kamani (*Terminalia catappa*). Of this total, 55 puparia were parasitized by only *Opius humilis*, 331 were parasitized by only *Diachasma tryoni*, 35 contained only larvæ of *D. fullawayi*, 96 contained living larvæ of *D. tryoni* together with dead larvæ of *O. humilis*, 4 contained dead larvæ of *D. tryoni* together with living larvæ

of *O. humilis*, 6 contained living larvæ of *D. fullawayi* together with dead larvæ of *O. humilis*, 1 contained a dead larva of *D. fullawayi* together with a living larva of *O. humilis*, and 90 were unparasitized. Here again is striking evidence of positive suppression of the parasite *O. humilis* by the other parasites, particularly by *D. tryoni*. Of the 618 puparia examined, the *O. humilis* occurred 107 times in combination with a species of Diachasma and won the struggle for existence in only 5 cases.

The foregoing would tend to explain the very noticeable fluctuations in the comparative abundance of the two principal species of parasites at differenct seasons of the year, as shown in Table I. It is seen that the extent of parasitism by *Opius humilis* is greatly influenced by the abundance or scarcity of the parasite *Diachasma tryoni*, and, as elsewhere discussed, the abundance of this latter parasite in Hawaii is very much dependent upon seasonal conditions. This causes a seasonal rise and fall in the effectiveness of *O. humilis*. In the summer and autumn of the year the ascendancy of the *D. tryoni* causes a great reduction in the abundance of *O. humilis*. During the winter and spring seasons the reduced activity of the Diachasma permits a rapid increase in parasitism by *O. humilis*. This is particularly true in the elevated Kona coffee district, where the winter temperatures are somewhat below those about Honolulu.

The results of the first series of dissections of fruit-fly larvæ and puparia on the island of Hawaii in January, 1916, led to further laboratory experiments in Honolulu on a comprehensive scale, duplicating as closely as possible the field conditions. Unparasitized fruit-fly larvæ were exposed within fruit to the attack of both *Opius humilis* and one or both of the species of Diachasma for a few hours. The fruit-fly puparia thus obtained were usually all parasitized, and no loss in time resulted from examinations of unparasitized material. In this manner 393 cases were obtained in which fruit-fly larvæ were parasitized by both *O. humilis* and *D. tryoni*. In 387 of these the *Opius* larvæ were all killed and the Diachasma survived. In only 5 of the puparia did the former succeed in overcoming the latter and developing.

Out of 77 cases in which fruit-fly larvæ were parasitized by both *Opius humilis* and *Diachasma fullawayi*, in only 2 did the Opius develop. In the remaining 75 the Opius larvæ were all killed by the Diachasma larvæ.

SUPPRESSION OF OPIUS HUMILIS BY TETRASTICHUS GIFFARDIANUS

The fruit-fly parasite *Tetrastichus giffardianus* Silvestri, a late introduction into Hawaii, has proved decidedly destructive to any of the opiines when occurring in the same fly larvæ or puparia with them. This chalcid, seemingly of importance, has, after a two years' trial in Hawaii, given but small promise of accomplishing any perceptible control of the

fruit fly. It is occasionally bred out from fruit-fly material secured in the field. Several times during dissections of puparia from the field its larvæ have been found in combination with larvæ of *Opius humilis* or of one of the species of Diachasma. Though soft, sluggish, and armed with small, inconspicuous, blunt mandibles, it nevertheless survives the opiine larvæ by sheer force of numbers and consequent rapid absorption of food. When ovipositing, this chalcid usually places about 10 eggs in the host at one insertion of the ovipositor. The opiines deposit but one egg, remove the ovipositor, and look for further larvæ to attack. A single oviposition, then, by a *T. giffardianus* into a host larva already parasitized by an opiine, places about 10 individuals of the chalcid with 1 of the opiine. If the host larva has already received several opiine ovipositions, usually only one individual is alive, as already shown. The larvæ of *T. giffardianus* exhibit no cannibalistic tendencies, and do not destroy each other. Many of the chalcid larvæ are then killed by the opiine larvæ, but seldom all of them. The opiines ultimately die, and one or more of the Tetrastichus larvæ develop. The death of the Opius or Diachasma larvæ results usually from starvation or suffocation or possibly by the absorption of toxic excretions of the Tetrastichus larvæ. Certainly the chalcid larvæ inflict no visible bodily injury on the opiine larvæ.

In view of the demonstrated ineffectiveness of this chalcid and of its capability for surviving the *Opius humilis* when occurring in fruit-fly larvæ with it, it is here considered a detrimental introduction because of interference with the work of the latter.

OPIUS HUMILIS, THE MOST PROLIFIC OF THE INTRODUCED PARASITES

From the foregoing summaries of the data it is obvious that the parasite *Opius humilis* is killed in the larva stage in almost every instance in which its larvæ become associated in a host larva with any one of the three other species of introduced fruit-fly parasites and that the percentage of cases of such duplication is large. Biological studies of all of these parasites, so far as two and three years' adaptation in a new country may show, have indicated quite clearly that under Hawaiian conditions *O. humilis* is the most prolific of the four species in all seasons of the year and that none of the others show particular abilities for reaching larvæ of the fruit fly that are not as easily accessible to the attack of *O. humilis*.

The very considerable activity of the parasite *Diachasma tryoni* and the occasional heavy parasitism by it has made necessary the establishment of careful proof that it is less prolific than *Opius humilis*, before it can be maintained that the introduction of the former is a detrimental one. The unimportance of the other two parasitic species eliminates all present need for discussing them any further.

From what has already been shown, we know positively that the parasite *Diachasma tryoni* has a clear field in Hawaii for unchecked

reproduction. *Opius humilis*, though present everywhere, does not hinder its activities. Extensive records kept in Hawaii during a period of three years on emergences from more than 100,000 fruit-fly puparia from many sections of the islands and from all available types of fruits have clearly shown that the average maximum degree of parasitism by *D. tryoni* does not exceed or equal that of *O. humilis*. This alone is sufficient proof of superior prolificness on the part of the latter.

In no instance have fruits of all varieties been found harboring larvæ of the fruit fly that are less frequented by *Opius humilis* than by *Diachasma tryoni*. The ability of the former to find the host is, then, equal, if not superior, to that of the other parasite. In no case has evidence been found to show that the longer ovipositor of the *D. tryoni* is really an advantage over that of the *O. humilis*, whose ovipositor is less than a third as long. The comparative difference is considerable, but the difference when considered in fractions of an inch is really small.

Preliminary records of individual females of both species to determine the total number of eggs deposited by an individual in a lifetime show no superiority of the *Diachasma tryoni* over *Opius humilis* in this respect.

The life cycle of *Diachasma tryoni* is consistently longer than that of *Opius humilis*. From 28,410 records on the length of the life cycle of the former, secured during 1916 and 1917, it is almost invariably found to be from two to four days longer than that of the latter during most of the year, and in the winter months a great number of the Diachasma individuals, hibernating in the larva stage, extended the cycle to from one to six months longer. From 22,889 cases under observation in 1916 and 1917 on the life cycle of *O. humilis*, in no single case has an individual ever been known to so hibernate or extend the length of the life cycle beyond the average for more than a few days. Cool weather and drouth seem most favorable for inducing this hibernating tendency in the larvæ of the *D. tryoni*. Of 3,077 cases under observation by the junior author in January, February, and March, 1917, in which fruit-fly larvæ had been parasitized by a Diachasma, a total of 1,404 cases occurred in which the parasite went into hibernation as a mature larva. This seems to explain the great reduction in abundance of *D. tryoni* in the field in winter. Its capacity as a parasite in the winter months is thus strikingly less than that of *O. humilis*.[1]

From the standpoint of longevity, all experiments so far show no great superiority of one species over the other, except as noted above in regard to hibernation. Individual adults of both *Opius humilis* and *Diachasma tryoni* have been kept alive for about four months.

[1] Credit is here due Mr. J. C. Bridwell for valuable suggestions offered in connection with the study of the hibernation of these parasites.

The relative proportion of females to males in these two species of parasites is interesting. Of 26,975 individuals of *Diachasma tryoni*, reared from material collected in the field in 1916 and 1917, 16,845, or 62.4 per cent, were males. Of 10,843 individuals of *Opius humilis* similarly reared from material collected in the field in 1916 and 1917, 6,128, or 56.5 per cent, were males. Here, again, the advantage, if any, lies with *O. humilis*.

In view of these several facts relating to the comparative prolificness of the parasites *Opius humilis* and *Diachasma tryoni*, it appears that the former is superior to the latter species or to any of the other introduced parasites. Thus, when a host larva is parasitized by both *O. humilis* and *D. tryoni*, the latter survives, and in so doing produces an individual less prolific than would have been the case had the *O. humilis* been permitted to develop. This seems to point to a certain, distinct loss. If one species of larval parasite when working alone parasitizes 60 per cent of the host, and another species not strikingly different from the first and working the same in every known respect parasitizes 40 per cent of the host when working alone, there is no reason to assume that both combined can exceed a parasitism of 60 per cent. All overlapping by the species capable of only 40 per cent parasitism can only serve to reduce the total effect to a point below 60 per cent of parasitism.

CONCLUSION

Sufficient evidence has been presented to prove the superiority of the parasite *Opius humilis* over the other introduced fruit-fly parasites in Hawaii and demonstrates the decided restraint operated over it by the unfailing cannibalistic activities of the larvæ of *Diachasma tryoni* in particular and of the other parasites in part. Knowing the capacity of *O. humilis* for parasitizing from 80 to 90 per cent of the larvæ of the fruit fly in favorable localities, such as the large Kona coffee belt on the island of Hawaii, the writers here maintain that detrimental results to a certain extent have arisen from the liberation in Hawaii of parasites other than *O. humilis* that attack the larva of the fruit fly. The total parasitism has simply been reduced in value to that of a parasite of secondary value.

It is hoped that the present analysis of the interrelated activities of the imported fruit-fly parasites in Hawaii may serve to stimulate greater discrimination in the selection of parasites proposed for future introduction.

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PLATE 10

Diachasma tryoni:

A.—Freshly hatched larva with its mandibles actually embedded in the body of a newly hatched but dead larva of *Opius humilis*. $\times$100.

B.—Newly hatched larva with its mandibles closed, showing ventral serosal material surrounding the body and the two gill-like appendages on the first body segment. $\times$ 100.

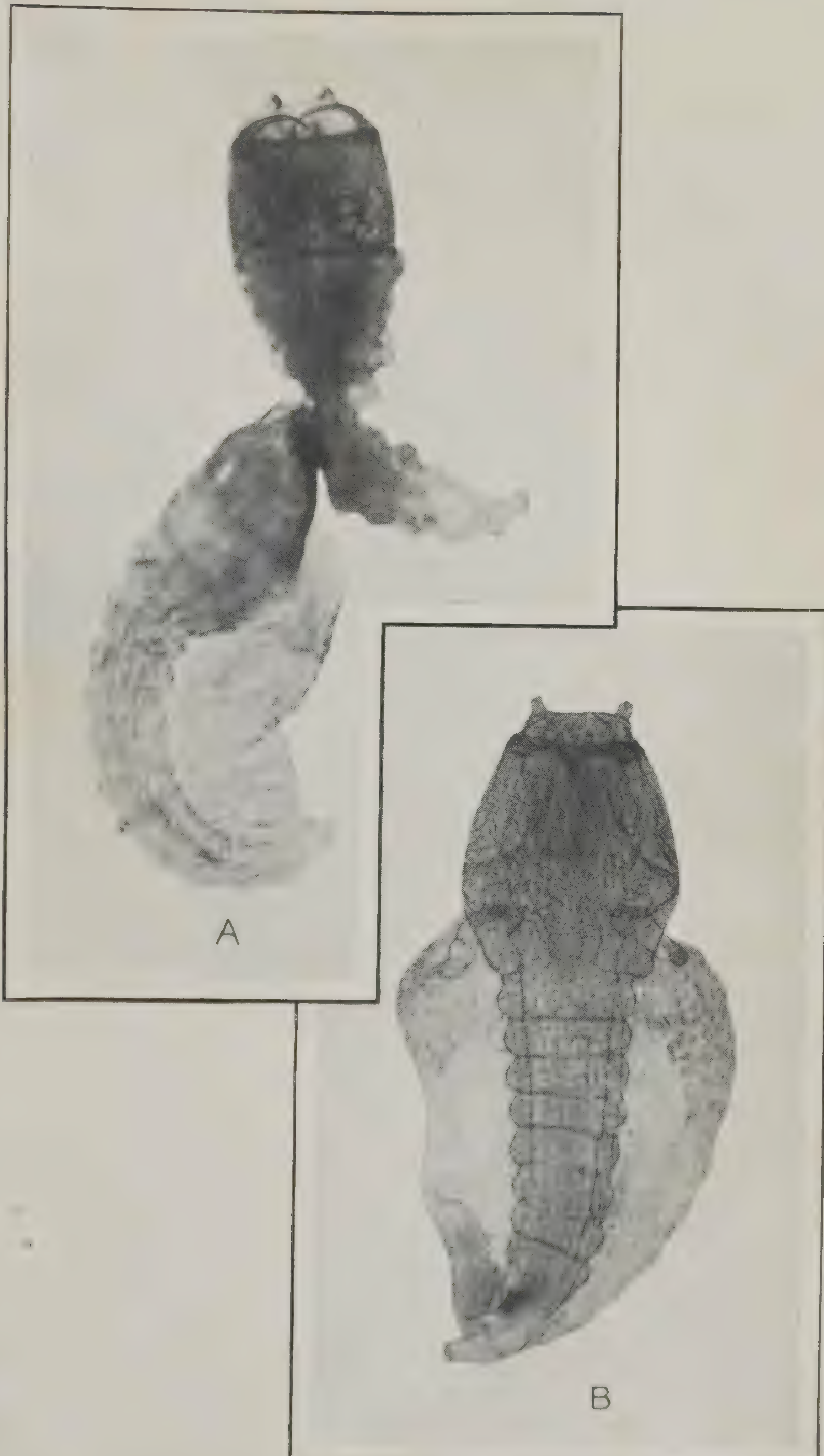

A
B

PLATE 11

Diachasma tryoni:

A.—Lateral view of larva in the second instar, showing particularly well the fat-body of the host recently taken in as food. × 100.

B.—Lateral view of a 2-day-old larva engorged with food and about to molt, showing the enlarged and stiffened body. × 100.

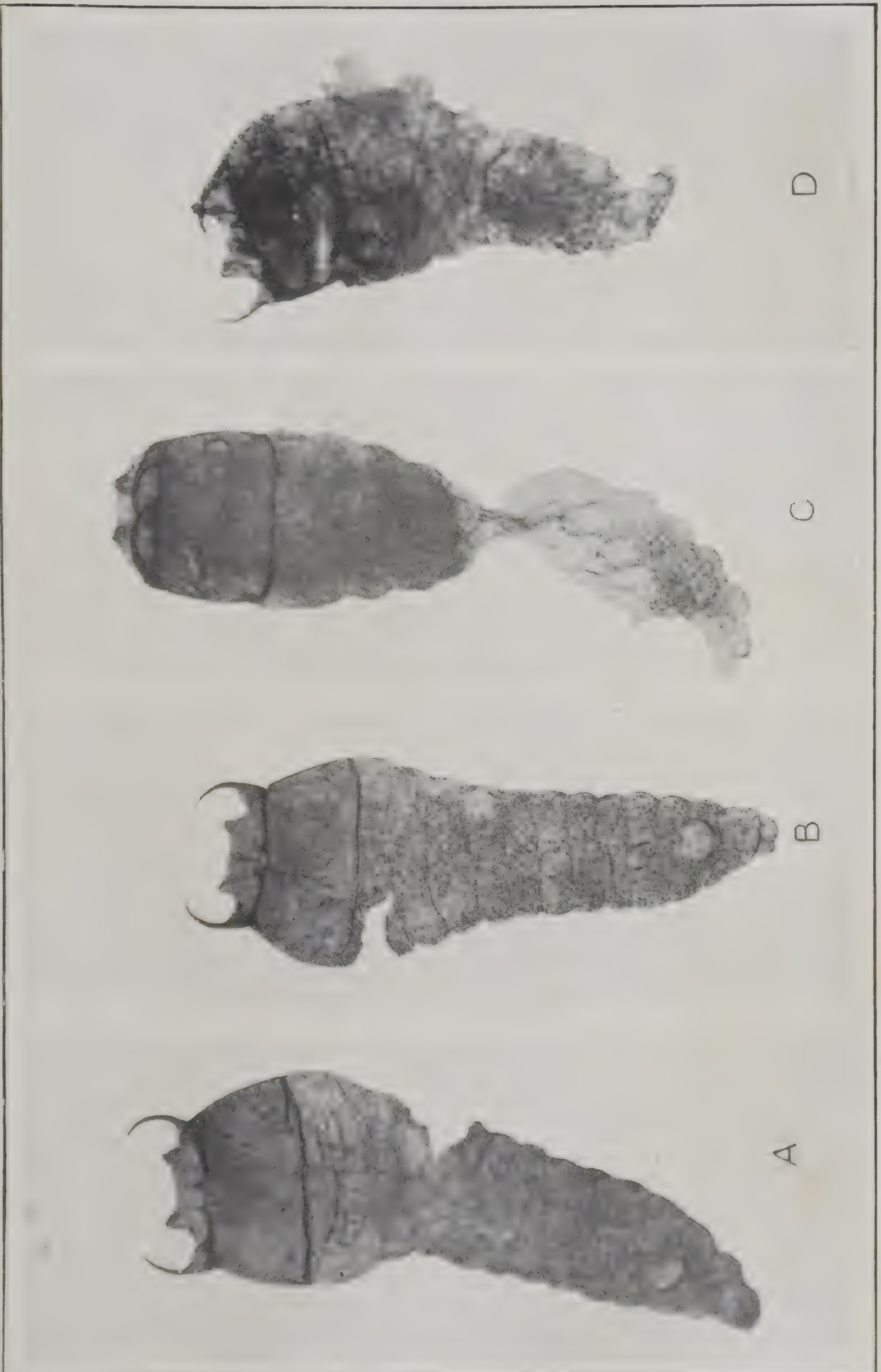
PLATE 12

Opius humilis:

A, B.—Dead larva in first instar; killed by first-stage larva of *Diachasma tryoni*, showing cut on body made by the attacking larva and mandibles extended in final death struggle. × 100.

C.—Dead larva in first instar; killed by first-stage larva of *Diachasma tryoni*. The point of attack is here clearly seen. The body contents have been apparently withdrawn from the lower portion of the body by the attacking larva. × 100.

D.—Dead larva in first instar; badly lacerated and distorted by attack of first-stage larva of *Diachasma tryoni*. In this case the dead larva was probably destroyed while n the embryonic stage and a few hours prior to the hatching of the egg. × 100.


A
B
C
D

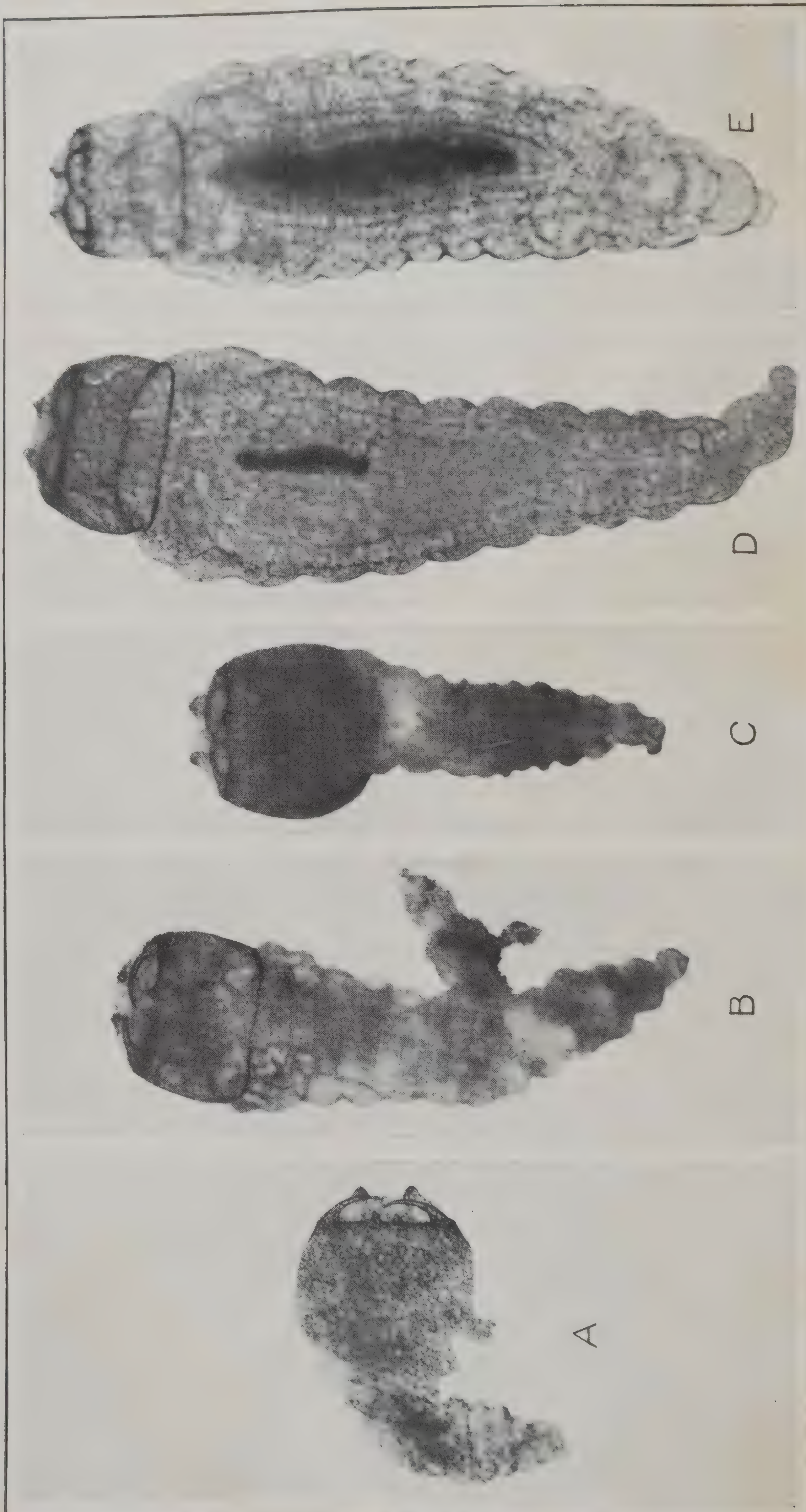

A
B
C
D
E

PLATE 13

Opius humilis:

A.—Dead larva in first instar, with body shriveled and twisted through attack by first-instar larva of *Diachasma tryoni*. × 100.

B.—Dead larva in first instar; killed by first-instar larva of *Diachasma tryoni*. Here the larva had been feeding and developing for several hours before being attacked by the Diachasma larva. The body contents can be seen protruding from an inflicted wound on the seventh and eighth body segments. × 100.

C.—Dead larva in first instar; killed by first-stage larva of *Diachasma tryoni*. This is the appearance of the dead Opius larvæ most commonly seen. The body is pinched by the mandibles of the Diachasma larva in the first or second body segment back of the head. × 100.

D.—Healthy, living larva in first instar. × 100.

E.—Healthy, uninjured, living larva in first instar. × 100.

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WATER EXTRACTIONS OF SOILS AS CRITERIA OF THEIR CROP-PRODUCING POWER

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THE PROBLEM

Given sunlight, suitable moisture and physical condition, the limitation on the power of soils to produce crops is variously ascribed to the following general causes:

1. Lack of capacity of the soil to supply the plant roots at all times with watery solutions of the essential elements in proper concentrations.
2. Presence of toxic substances.
3. Lack of physiological balance in dissolved soil constituents.

It is clear that any one or a combination of these conditions may be the cause of low production, even though the remaining conditions may be entirely satisfactory. The instances where the second and third inhibitive causes operate to limit production are perhaps quite numerous. It may even be that, because of frequent occurrence, they are of as much or more practical importance than incapacity of the soil to supply the plant with proper nutrients. Nevertheless we are disposed to regard such instances as special cases and to lay greater stress on the capacity of the soil to deliver up solutes to the growing plant. Studies involving this capacity are obviously more fundamental, in that they have to do with an important function of all soils.

WATER-EXTRACTABLE SUBSTANCES IN SOILS

When we consider this function, we naturally turn to water as the logical agent for the removal and determination of the soluble substances in soils. The application of water as a solvent requires the use of a sufficient amount in proportion to the soil to cause it to come into equilibrium with the true soil solution and thus to insure the complete removal of all dissolved matters when the soil suspension is filtered. Water in excess unquestionably removes not only the substances already in solution but additional quantities somewhat proportional to the amount of water used.[1] Furthermore, we are told that the absorption of solutes by plants is related to the concentration of the soil solution and not to

[1] HOAGLAND, D. R. THE FREEZING-POINT METHOD AS AN INDEX OF VARIATIONS IN THE SOIL SOLUTION DUE TO SEASON AND CROP GROWTH. *In* Jour. Agr. Research, v. 12, no. 6, pp. 369-395, 9 fig. 1918.

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the absolute amounts present in the soil. We heartily concur in this opinion; but the impossibility of removing for investigation the true and unmodified soil solution, even from soils whose optimum water content is high, is too well recognized to require discussion. It is true that by the method of Bouyoucos and McCool [1] we can determine the total concentration of the soil solution, but are left in the dark as to the concentration of the individual solutes in that solution.

In the present paper, therefore, our data are presented in terms of the amounts of solutes extracted from soils and not in terms of concentrations. We shall endeavor to use this material in such a way as to justify certain conclusions as to the presence or absence of power on the part of soils to supply the needs of crops and to maintain optimum concentrations in the soil solution. Hoagland,[2] in this laboratory, has shown that, while the total amounts of material extracted from soils by an excess of water are invariably greater than those contained in the soil solution, they are of the same general order of magnitude (as 2 is to 4 or 5), depending on the type of soil. Obviously, then, if the amounts of soil constitutents extracted from cultivated soils can be shown to be relatively high or relatively low, we may legitimately infer that the amounts in the soil solution are correspondingly high or low. We do not mean to imply that figures so obtained would necessarily indicate the existence of an adequate supply of any element, because even low figures might constitute adequacy. The question of adequacy or inadequacy may or may not be answered by such data, but it would certainly reflect the relative magnitudes of the present supply although it might not indicate the soils' power of renewal.

Data presented by Stewart [3] in figures 8 to 20 show that normal seasonal fluctuations in the amounts of the essential elements extracted at different times by water (1 to 5) from a cultivated soil (cropped or uncropped) are likely to be as great as the variations between different soils. He also shows that soils under crop (barley) invariably contain smaller amounts of nitrate, potassium, calcium, and magnesium than their uncropped duplicates, apparently reflecting the inability of soils to maintain their initial concentrations and at the same time supply the needs of growing plants. Since the fluctuations in the amounts of water-soluble substances in soils are known to be quite large and the effect of withdrawal important, it follows that the limitations of soils can best be shown by data obtained during the period in which the withdrawal is actually taking place.

A comparison of the charts referred to above shows that, even though good (uncropped) soils may contain considerably more solutes than poor

[1] Bouyoucos, G. J., and McCool, M. M. The freezing point method as a new means of measuring the concentration of the soil solution directly in the soil. Mich. Agr. Exp. Sta. Tech Bul. 24, p. 592–631, 2 fig. 1916.

[2] Hoagland, D. R. Op. cit.

[3] Stewart, G. R. The effect of season and crop growth in modifying the soil extract. *In* Jour. Agr. Research, v. 12, no. 6, pp. 311–368, 24 fig., pl. 1918.

(uncropped) soils, both good and poor soils are reduced to the same general level at the time the crop is growing. The condition of soils under crop is epitomized in Table I.

TABLE I.—*Water extractable matters in soils[a] under crop. Average during period[b] of low nitrate content*

[Results expressed as parts per million of soil]

	Productivity.												
	Good.							Medium.			Poor.		
Soil No.	1	2	5	6	8	11	14	4	7	10	3	9	12
Nitrate (NO_3)	5	5	4	5	5	4	4	6	5	8	5	5	5
Phosphate (PO_4)	7	5	17	7	12	23	11	9	7	13	6	7	13
Basic ions ($K+Ca+Mg$)	65	71	108	54	62	72	76	130	69	45	64	54	41

[a] For a detailed description of soils, see STEWART, G. R. Op. cit.
[b] Period covered: Soil 7, twelfth to eighteenth week after planting; soils 12 and 14, eighth to eighteenth week after planting; all others, tenth to eighteenth week after planting.

We observe in Table I a general uniformity in the magnitudes of the various solutes present at the period of depletion in soils of varying productive capacity. Apparently crops first reduce the supply of solutes until these approach a comparatively low and (with a few exceptions) fairly uniform level. Subsequent withdrawals must further reduce the existing supply or be made good by new material coming into solution.

WITHDRAWAL BY CROPS

In the second year's experiments reported by Stewart we installed eight additional containers of soil 1, planted to barley, and harvested and analyzed the crop from time to time. This particular soil was chosen because it was known to be one of our most productive soils and the amounts withdrawn by the crop would presumably represent the usual draft to be expected from the crop under consideration. The data obtained are presented in Tables II and III. In scrutinizing these data it must be remembered that we are not attempting to show the requirements of plants or their nutritive habits, but merely their effect on the soil.

If we now compare these data with the water-soluble content of the soil, we may develop the approximate relation between the demand of barley and the supply in a productive soil. The relation is expressed in the column headed "Ratio." Literally interpreted, the ratio for each ion expresses the number of day's supply contained in the water extract during the several periods covered. Thus, in the case of nitrate we see that there was never less than a nine days' supply in the soil during any period of the growing season. If the plant is capable of absorbing all of

the nitrate present in the water-soluble condition, we have direct evidence that the water-soluble nitrate need only be renewed once in nine days even at this critical period, while at all preceding and subsequent periods the rate of renewal may be much slower. If we apply this same reasoning to all of the ions, we develop the fact that there are always present in this particular soil, in a condition capable of solution in water, at least—

Nine days' supply of nitrate.
Twenty-five days' supply of phosphate.
One hundred and forty-four days' supply of potassium.
Two hundred and sixty-five days' supply of calcium.
Seventy-six days' supply of magnesium.

TABLE II.—*Rate of withdrawal by crop [a] as shown by periodic harvesting of soil 1*

[Withdrawals computed to parts per million of soil—50 plants and 1,800 pounds of soil to the unit container]

Date.	Nitrate (NO_3).[b]			Phosphate (PO_4).[b]			Potassium (K).		
	Grams per plant.	Parts per million of soil.		Grams per plant.	Parts per million of soil.		Grams per plant.	Parts per million of soil.	
		Entire.	Per day.		Entire.	Per day.		Entire.	Per day.
May 1 (planted)	0.000	......	......	0.000	......	......	0.000	......	......
June 12	.405	24.85	0.59	.038	2.32	0.055	.133	8.13	0.194
June 26	(?)	......	......	.115	7.02	.336	(?)	......	......
July 12	.520	31.90	.25	.120	7.35	.024	(?)	......	......
July 24	.594	36.40	.32	.132	8.10	.054	.223	13.70	.133
August 7	.629	38.40	.14	.144	8.82	.051	.233	14.30	.043
August 21	.664	40.80	.17	.184	11.20	.170	.276	16.90	.186
August 28	.694	42.50	.24	.194	11.90	.100	.294	18.00	.157

Date.	Calcium (Ca).			Magnesium (Mg).		
	Grams per plant.	Parts per million of soil.		Grams per plant.	Parts per million of soil.	
		Entire.	Per day.		Entire.	Per day.
May 1 (planted)	0.000	....	....	0.000	....	....
June 12	.012	0.712	0.017	.013	0.816	0.019
June 26	.034	2.092	.098	.031	1.920	.079
July 12	(?)	....	....	.036	2.190	.019
July 24	.041	2.530	.016	(?)	....	....
August 7	(?)	....	....	.044	2.670	.017
August 21	.048	2.940	.014	.049	2.980	.022
August 28	.043	2.650	....	.051	3.140	.023

[a] Analyses based on varying numbers of plants: June 12, 69 plants; June 26, 42 plants; July 12, 42 plants; July 24, 29 plants; August 7, 28 plants; August 21, 38 plants; August 28, 87 plants.
[b] Nitrate (NO_3) and phosphate (PO_4) computed from the amounts of nitrogen and phosphorus in the crop.

The entire time the crop was in the ground was 119 days. The time to elapse after the period of greatest depletion was 35 days for nitrate, 63 days for phosphate, 77 days for potassium, 63 days for calcium, and 63

days for magnesium. It would appear that there is actually present in the water-soluble condition at the period of greatest depletion more than enough potassium, calcium, and magnesium to supply the entire subsequent withdrawals of the crop at the same rate, and that any renewal or increase of these amounts should be unnecessary if the plant is capable of utilizing the entire supply present.

TABLE III.—*Comparison of water-extractable matters in soil 1 (cropped) with daily draft by plant*

Nitrate (NO_3).				Phosphate (PO_4).				Potassium (K).			
Weeks.	Parts per million of soil.		Ratio.	Weeks.	Parts per million of soil.		Ratio.	Weeks.	Parts per million of soil.		Ratio.
	In soil.	Daily draft.			In soil.	Daily draft.			In soil.	Daily draft.	
1-6, inclusive	69	0.59	117	1-6, inclusive	7	0.055	127	1-6, inclusive	28	0.194	144
7-10, inclusive	4	.25	16	7-8, inclusive	8	.336	24	7-12, inclusive	29	.133	217
11-12, inclusive	3	.32	9	9-10, inclusive	10	.024	417	13-14, inclusive	34	.043	790
13-14, inclusive	8	.14	57	11-12, inclusive	3	.054	55	15-16, inclusive	38	.186	204
14-16, inclusive	3	.17	18	13-14, inclusive	8	.051	157	17th	....	.157	....
17th	....	.24	....	15-16, inclusive	5	.170	29				
				17th	....	.100	....				

Calcium (Ca).				Magnesium (Mg).			
Weeks.	Parts per million of soil.		Ratio.	Weeks.	Parts per million of soil.		Ratio.
	In soil.	Daily draft.			In soil.	Daily draft.	
1-6, inclusive	28	0.017	1,647	1-6, inclusive	15	0.019	789
7-8, inclusive	26	.098	265	7-8, inclusive	6	.079	76
9-12, inclusive	18	.016	1,125	9-10, inclusive	14	.019	736
13-16, inclusive	21	.014	1,500	11-14, inclusive	9	.017	529
17th	......	......	....	15-16, inclusive	20	.022	909
				17th	......	.023	....

It is evident that nitrate and phosphate are in a class by themselves, in that the supply must be renewed at a much more rapid rate than any of the other ions; but, inasmuch as this soil was highly productive, it must have possessed the power to replenish the soil solution as required. But we have shown heretofore that there is very little difference in the absolute amounts of solutes between good and poor soils under a crop of barley. It follows that figures of the same order of magnitude as those just stated would be obtained by similar computations from the poor soils. We conclude, therefore, that in soils in any degree fit for agricultural purposes (with the possible exception of very abnormal types, such as peat, etc.), the potentially soluble matters are sufficient in amount to meet the requirements of crops; but it does not follow that the plant is capable of drawing upon this supply at the concentrations corresponding to these amounts.

CONCENTRATION AND RATE OF FORMATION OF SOLUTES

Since even our poor soils appear to be able to furnish adequate amounts of all solutes at the most critical periods, we can only ascribe nutritive difficulties of soils to inability on the part of the plant to absorb this potentially soluble supply. The rate of absorption by any plant may be limited by the possible supply and by the concentration of that supply, but we have seen that in all cases which have come under our observation the supply is adequate at all times. We should therefore either measure the concentration of the soil solution itself or evolve some other means of estimating the relative power of soils to maintain an optimum concentration. Our data do not permit us, unfortunately, to estimate the concentration of individual ions in the soil solution, but we may perhaps get an idea of the relative power of soils to maintain or restore a proper concentration by consideration of the data on soils with and without crop. We present in Table IV the seasonal averages of the water-extractable substances in cropped and uncropped soils.

TABLE IV.—*Water-extractable matters in cropped and uncropped soils. Seasonal averages*

[Results expressed as parts per million of soil]

Constituent.	Productivity.							
	Good.							Average all.
Yield in bushels per acre	86.5	84.0	88.4	85.9	87.0	80.1	80.8	84.7
Soil No.	1	2	5	6	8	11	14	
Nitrate:								
Uncropped	131	120	146	141	180	154	96	137
Cropped	36	33	30	43	28	27	23	30
Difference	95	87	116	98	152	127	73	107
Phosphate:								
Uncropped	7	5	18	8	17	30	13	14
Cropped	6	5	17	8	12	26	11	12
Difference	1	0	1	0	5	4	2	2
Potassium:								
Uncropped	40	55	47	33	49	67	75	52
Cropped	31	50	33	27	31	48	59	40
Difference	9	5	14	6	18	19	16	12
Calcium:								
Uncropped	34	37	91	47	66	51	32	51
Cropped	24	19	58	28	34	29	20	30
Difference	10	18	33	19	32	22	12	21
Magnesium:								
Uncropped	23	15	24	21	15	13	6	17
Cropped	13	7	17	10	10	7	4	10
Difference	10	8	7	11	5	6	2	7

TABLE IV.—*Water-extractable matters in cropped and uncropped soils. Seasonal averages*—Continued

Constituent.	Productivity.							
	Medium.			Average all.	Poor.			Average all.
Yield in bushels per acre	70.4	69.4	70.6	70.1	56.2	45.8	50.0	50.7
Soil No.	4	7	10		3	9	12	
Nitrate:								
Uncropped	141	118	130	129	88	54	83	75
Cropped	33	34	39	35	30	18	23	24
Difference	108	84	91	94	58	36	60	51
Phosphate:								
Uncropped	11	7	11	9	7	6	13	9
Cropped	10	7	13	10	6	6	12	8
Difference	1	0	−2	−1	1	0	1	1
Potassium:								
Uncropped	45	31	49	42	43	19	30	31
Cropped	40	22	30	31	37	18	20	25
Difference	5	9	19	11	6	1	10	6
Calcium:								
Uncropped	84	61	52	66	32	44	41	39
Cropped	66	41	25	44	25	35	24	28
Difference	18	20	27	22	7	9	17	11
Magnesium:								
Uncropped	26	13	11	16	14	9	9	10
Cropped	22	10	6	12	11	8	6	8
Difference	4	3	5	4	3	1	3	2

EXAMINATION OF UNCROPPED SOILS.—We regard the magnitudes shown as the resultant of the combined effects of previous withdrawals, the time which has elapsed since the soil was last depleted, and the rate at which the soil is capable of replacing solutes removed from the soil solution. A soil might show high figures simply because previous withdrawals by crops had been relatively low or remote in time. Nevertheless it is significant that the average content of the more productive soils for each constituent is relatively considerably higher than the average for the poor soils. We infer from this that high figures ordinarily, but not necessarily, indicate a relatively high rate of elaboration of solutes on the part of the soil. A conspicuous example of an exception to this would be the case of a so-called alkali soil, where large accumulations of solutes might have no relation to the present elaborate power of the soil.

EXAMINATIONS OF CROPPED SOILS.—While all averages of the good soils under crop are higher than the averages of the poor soils, the magnitudes of the differences are quite small. Apparently the crop by withdrawal or indirect effects on the soil tends to reduce the initial amounts of solutes

(and presumably their concentrations) to the same general level in both good and poor soils. It seems probable that, when certain minima are reached, the plant can not absorb solutes, and subsequent withdrawal then depends upon the capacity of the soil to elaborate additional solutes as rapidly as the plant requires them. The figures themselves, however, can not give a direct measure of this capacity, but small variations may possibly reflect important differences in the rate at which solutes are elaborated by different soils.

Equivalence of bases.—If we compare the figures for the individual basic ions in good and poor soils (both in the cropped and uncropped condition), we find numerous instances in which some one or more ions are lower in the good soils than in the poor soils. These discrepancies need not concern us if we regard the figures merely as a means of getting at the power of soils to replenish the soil solution. In fact, this power is more likely to be reflected by the aggregated soluble matters than by individual constituents, and preferably by the aggregate of those constituents of similar chemical properties. We propose, therefore, to consolidate our data for the basic ions in one figure (by addition) for subsequent consideration. If we further consider all of our data with reference to the seasonal requirements of a good crop, we can perhaps indicate why some soils are more productive than others.

In Table II we gave the amounts of the chemical elements found in a good crop of barley produced on soil 1, computed to parts per million of soil. These figures were 42.50 p. p. m. of nitrate, 11.90 p. p. m. of phosphate, 18.00 p. p. m. of potassium, 2.9 p. p. m. of calcium, and 2.98 p. p. m. of magnesium; or in round numbers, 42 p. p. m. of nitrate, 12 p. p. m. of phosphate, and 24 p. p. m. of total bases. We assume these figures to represent the probable requirements of a good crop of barley on all soils and propose to examine our data with reference to the capacity of the various soils to meet this requirement. In Table V we combine all pertinent data available.

Nitrate.—The figures for excess in the good and medium soils show an extraordinary discrepancy between the amounts of nitrate in the uncropped soil and the amounts found in the cropped soil plus the withdrawal of a good crop. We can not satisfactorily account for this discrepancy, which appears to be caused in part by an inhibition of nitrification due to the presence of the crop, but which also represents an actual loss of nitrate from the cropped soil. Since the soils were kept in tight containers, there could be no loss from drainage; and neither denitrification, reduction to ammonia, nor the possible loss of ammonia by way of the plant appeals to us as an adequate explanation. We can only suggest that the presence of a crop may cause such a change in the biological environment of the soil that the nitrogen of nitrates is stored in insoluble (protein) forms in the soil. For our present purposes, however, it is only necessary to point out that such losses appear to be a necessary

incident to the production of a good crop. If the soil is not capable of sustaining such losses, it is extremely probable that crops will suffer from a lack of nitrates.

TABLE V.—*Water-extractable matters with reference to seasonal withdrawals of a good crop*

[Expressed as parts per million of soil]

Constituent.	Productivity.												
	Good.							Medium.			Poor.		
Soil No.	1	2	5	6	8	11	14	4	7	10	3	9	12
Nitrate (NO_3)													
(*a*) In cropped soil	36	33	30	43	28	27	23	33	34	39	30	18	23
(*b*) In a good crop	42	42	42	42	42	42	42	42	42	42	42	42	42
(*c*) Sum ($a+b$)	78	75	72	85	70	69	65	75	76	81	72	60	65
(*d*) In uncropped soil	131	120	146	141	180	154	96	141	118	130	88	54	83
Excess ($d-c$)	63	55	74	56	110	85	31	66	42	49	16	−6	18
Phosphate (PO_4):													
(*a*) In cropped soil	6	5	17	8	12	26	11	10	7	13	6	6	12
(*b*) In a good crop	12	12	12	12	12	12	12	12	12	12	12	12	12
(*c*) Sum ($a+b$)	18	17	29	20	24	38	23	22	19	25	18	18	24
(*d*) In uncropped soil	7	5	18	8	17	30	13	11	7	11	7	6	13
Deficiency ($c-d$)	11	12	11	12	7	8	10	11	12	14	11	12	11
Bases (K Ca Mg):													
(*a*) In cropped soil	68	76	108	65	75	84	83	128	73	61	73	61	50
(*b*) In a good crop	24	24	24	24	24	24	24	24	24	24	24	24	24
(*c*) Sum ($a+b$)	92	100	132	89	99	108	107	152	97	85	97	85	74
(*d*) In uncropped soil	97	107	162	101	130	131	113	155	105	112	89	72	80
Excess ($d-c$)	5	7	30	12	31	23	6	3	8	27	−8	−13	6

When we turn to the poor soils, we see that they all had in the uncropped condition more than enough nitrate to supply the actual amounts withdrawn by a good crop; but all were incapable of sustaining additional losses of the magnitudes, which we must regard as normal.

PHOSPHATE.—The figures for all soils under crop plus the requirements of a good crop are invariably greater than the amounts in the uncropped soils. If the figures for these latter represent in each case the maximum amount of soluble phosphate the soil is able to hold in that condition, it is quite clear that either the plant absorbs insoluble phosphate, or the good soils replace the soluble phosphate as rapidly as it is required by the plant. The latter explanation appears the more probable. But the computed deficiencies of the poor soils are no greater than those of the good soils, nor are the soluble phosphate contents of the former less than many of the latter. The rate of solution of phosphate in the good soils must be very high and we can find no reason to conclude that the poor soils are in any way inferior in this respect.

BASIC IONS.—The amounts of basic ions in cropped soils plus the requirements of a good crop are usually less than the amounts in the uncropped soils. We note two exceptions to this in the poor soils Nos.

3 and 9. For these soils to have supplied the requirements of a good crop and at the same time to have maintained their general level of concentration would have necessitated that more bases come into solution. It is not impossible that they would have responded to the demand for bases, just as they would probably have responded to the demand for phosphate. The important difference between these poor soils and the remaining soils is that the latter would never have been called upon to furnish larger amounts of bases than the uncropped soil shows ability to supply.

CRITERIA OF FERTILITY

The growth of a crop reduces the average nitrate content of soils to a comparatively uniform level in soils of all degrees of productivity (see Tables IV and V). The figures for nitrate in uncropped soils are always higher than the known withdrawals by plants, but may not always be sufficiently high to supply these withdrawals and certain other inherent losses. The relative ability of soils to meet these losses may be inferred from the amounts of nitrate in the uncropped soils.

Except in a few soils containing large amounts of soluble phosphate, the growth of a crop does not reduce this ion to any considerable extent. (The small differences shown approach the magnitude of experimental error.) Furthermore, the figures do not show that good soils possess the power of renewing the soluble phosphate more rapidly than the poor soils. Figures for soluble phosphate can not be considered to reflect the relative power of soils to supply the plant.

The growth of a crop reduces the basic-ion content of soils, but the amounts remaining are still far in excess of the crop requirements. It is not improbable, however, that their concentrations are in some cases falling below the optimum requirements of plants. If this be the case, comparison of the basic solutes of cropped soils may indicate deficiency in this respect. The differences between the basic ion contents of cropped and of uncropped soils are usually, but not always, greater than the demands of crops. These differences therefore express the relative power of soils to supply the crop requirements and to maintain the concentration equivalent to the amounts shown by the cropped soil, without drawing upon greater amounts of solutes than the uncropped soil indicates capacity to supply.

Test of criteria.—If we arrange our soils in the order in which they possess the various characters to which attention has been called, we may be able to bring out salient differences between good and poor soils. The order in which the soils occur in each character is frequently determined by very small variations in absolute amount of solutes, but it must be remembered that these are based on considerable numbers of analytical determinations and are probably not vitiated by experimental errors.[1]

[1] Stewart, G. R. Op. cit.

Very small differences at critical points may reflect significant differences in the performance of soils (fig. 1).

All of the poor soils appear among the three lowest in two out of three characters. If we examine the medium soils, we find that Nos. 4 and 10 are among the three lowest in one character. If we draw a line over the three lowest characters as a tentative indicator of the existence of sub-optimal conditions, we find that five of our six medium and poor soils fall below in one or more characters; that the remaining medium soil, No. 7, approaches it in one character; that none of the good soils fall below in any character. It is true that several of the good soils, No. 14, 6, and 1, approach the line very closely, and we do not wish to be understood as attaching too much importance to slight differences in the

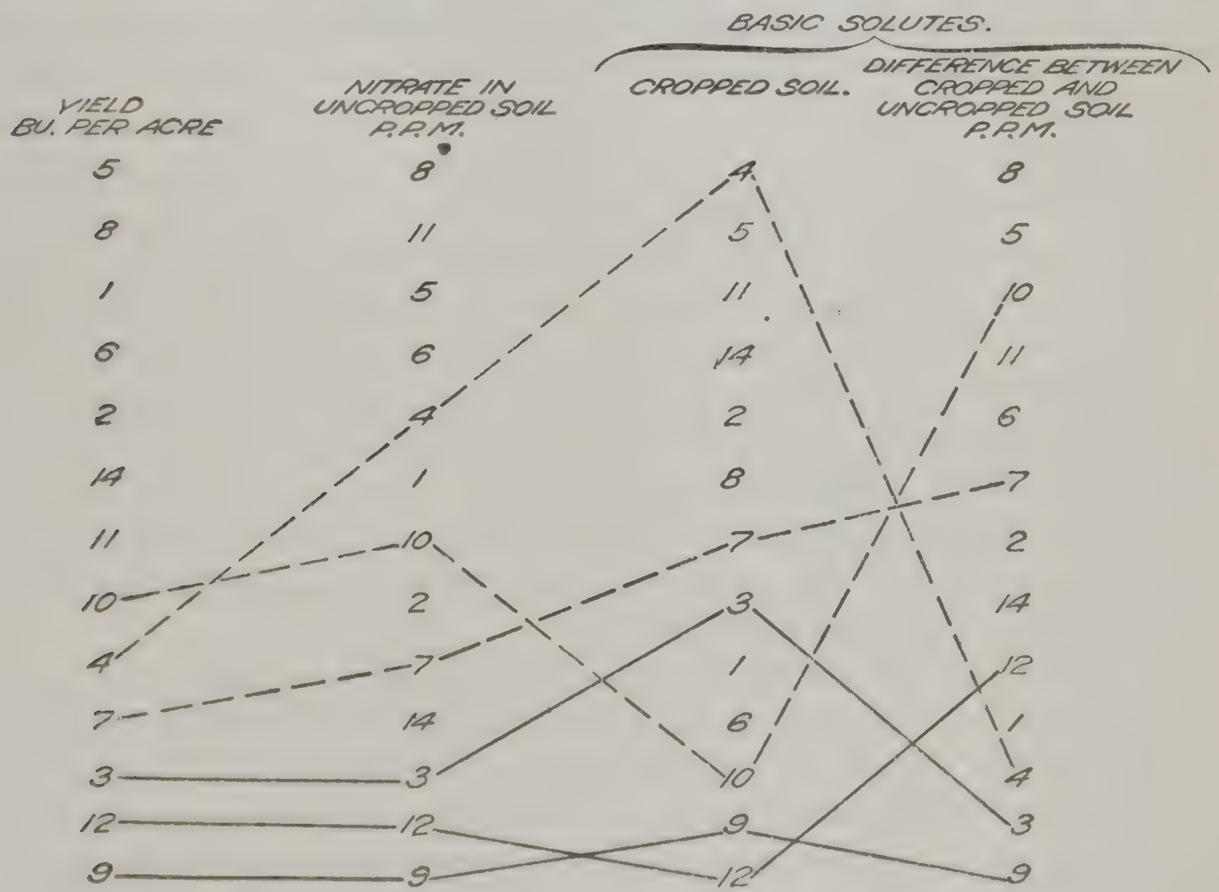

FIG. 1.—Graphs showing soils arranged with reference to yield and important characters.

magnitudes of these characters. It would appear, however, that the yields of soils 7, 3, 12, and 9 are closely correlated with their nitrate content, but the mediocre yields of soils 4 and 10 can only be accounted for by a reference to other characters.

We conclude that the nitrate content of uncropped soils is the most valuable single criterion for appraising the crop-producing power of soils; that the amount of basic ions in cropped soils is indicative of the extent to which soils tend to maintain their concentrations when subjected to depletion by crops; that the differences in basic-ion content between cropped and uncropped soils may reflect the ability of the soil to meet the demands of the crop without dangerous diminution of concentration and without drawing upon the reserve (insoluble) supply of the soil.

CONCLUSIONS

The evidence presented in the early part of this paper indicates that there is always present in soils, in a condition permitting ready solution in water, enough of the more important chemical elements to supply the immediate needs of crops. It is hardly conceivable that substances in this condition do not represent a potentially available supply. Inasmuch as this supply never entirely disappears, even in the case of nitrates, it would seem that there is no such thing as a lack of available nutrients in soils which are at all productive, but that a plant may still be unable to satisfy its requirements if the concentration with reference to the individual ions falls below certain minima. It is furthermore highly probable that the optimum concentration varies with every soil in accordance with the physicochemical system present in the soil solution. Slight differences in the character of this system may modify in a marked degree the power of a plant to absorb solutes, so that even if we were able to obtain and analyze the true soil solution, we would not necessarily be able to say that any figure for individual ions constituted inadequacy.

Attention has been called to certain characters as reflecting the composition of the soil and its power to produce crops. Inasmuch as these involve three variables to which it is impossible to assign definite relative weights, an exact correlation of productivity with the figures presented is not to be expected. Nevertheless, the correlation between the general magnitudes of the figures presented and the crop-producing powers of the soils studied is sufficiently close to justify the belief that they give expression to the relative power of soils to produce crops, although they are not an exact measure of that power.

We believe that the evidence obtained is sufficient to justify the hope that we may be able to predict, within reasonable limits, the relative crop-producing powers of soils by comparing their figures expressing these characters with similar data derived from soils whose productive power is known. Before such a method is generally applicable, however, it will be necessary to study the behavior of many soils with numerous type crops. This is quite feasible if the various characters can be developed without the enormous number of analytical determinations involved in the present experiments. We believe that this can be accomplished by substituting for our figures, representing the sums of the basic ions, figures obtained for total soluble salts, or preferably direct determinations of the concentration of the soil solution by some such method as that presented in the preceding paper.[1] It is quite certain that we shall never have a precise measure of soil fertility until soils are studied concurrently in the cropped and the uncropped condition and under strict control. The reasons are obvious in that data from the soil under crop can not indicate its latent power, and data

[1] HOAGLAND, D. R. Op. cit.

from the uncropped soil taken alone do not take into account the fact that the solutes in the cropped soils can not be reduced below certain minimum and probably variable limits.

In the present paper we have dealt entirely with chemical criteria because they afford the most convenient expression of the results of the activities of the soil, chemical, physical, and biological. We do not wish to be understood as minimizing the importance of biological studies because we regard living organisms as being the most important single agency, through the formation of nitrates and carbonic acid, in modifying the soil solution. While biological studies have a most important bearing on the proper treatment of soils, the resultant effects of all activities upon these heterogeneous mixtures can only be developed by the actual growth of crops and observation of their effects.

EFFECT OF SEASON AND CROP GROWTH IN MODIFYING THE SOIL EXTRACT

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HISTORICAL REVIEW

The first studies on the water-soluble material of soils were inspired by the results of the investigations of the absorption of plant nutrients carried on by Thompson (55)[2] and by Way (*60*). Their epoch-making discoveries in the year 1850 aroused a widespread discussion of the manner in which the essential compounds might be held in the soil.

Liebig (*39*) was greatly impressed by this work and carried out a series of investigations in which he studied the absorption of calcium phosphate and potassium sulphate. He concluded that, since the phosphate and potassium radicals were so readily absorbed by soils, very little of these essential nutrients could be present in the soil water. The results of lysimeter and drainage studies confirmed him in his belief, and he proposed the theory that the plant roots must be able to draw nourishment directly from the soil particles. At the same time Grouven (*22*) had analyzed the extract from three soils obtained by percolating 6,000 c. c. of distilled water through 2,000 gm. of soil. He attempted to relate these figures to the amount of material that would be brought into solution by the season's rainfall.

Eichhorn (*14*) also studied a soil near Bonn, Germany, in an attempt to obtain a solution which would approximate the moisture existing in the soil. He added 36.5 per cent by weight of water, and allowed it to stay in contact with the soil for 10 days. He concluded that the soil contained all water-soluble nutrients necessary for raising a crop.

This view was severely criticized by Wunder (*63*), who held with Liebig (*39*) that the soil could not furnish sufficient water-soluble material. Schumacher (*52*) replied to Wunder (*63*), upholding Eichhorn's views. Gradually, through the work of Peters (*45*), Jarriges (*31*), Ulbricht (*58*), Hoffman (*28*), Wolff (*62*), and Cossa (*13*), it became evident that the water extract from soils contained the major plant nutrients.

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[1] The writer desires to make acknowledgment of the assistance of the Division of Soil Technology, of the California Experiment Station, in selecting the soils employed in the investigation and in performing the physical analyses reported in Table II. The writer is also indebted to Mr. A. W. Christie, of this station, for the performance of ammonification and nitrification studies and to Messrs. H. E. Billings, A. W. Christie, and J. C. Martin, of this Station, for assistance in portions of the analytical work performed in 1916.

[2] Reference is made by number (italic) to "Literature cited," p. 364–368.

The existence of appreciable quantities of water-soluble phosphates was the point longest in doubt, though the results of Heyden (*25*) and Schulze (*51*) established this fact satisfactorily.

The most notable of the early investigations and those which have had the greatest influence on modern work were performed by Schlœsing (*46, 47*). His method consisted of treating 30 to 35 kgm. of soil with an artificial rain and then collecting the first portions of clear solution which ran through. This he believed to be identical with the actual soil solution, and his was the first attempt to obtain it in an unaltered condition. Schlœsing showed the presence of all the principal elements in this soil extract, and his procedure is still occasionally used in European work. Schlœsing, jr. (*48, 49*), has continued this portion of his father's work, devoting special attention to water-soluble phosphates, and has concluded that there are differences in the phosphate content of various soils, and also that there is almost enough soluble phosphate present in most soils to supply an average crop.

The first work performed in the United States on the water-soluble material of soils was that undertaken by King (*36–38*), and his coworkers at Wisconsin. This was largely devoted to studies of the nitrate content of cultivated field soils, and was later extended to studies of the total salt content by the use of conductivity methods. The results obtained in this work may be considered to have been merely preliminary to King's more extensive investigations performed in the Bureau of Soils of the United States Department of Agriculture (*34, 35*).

Before this later work of King's appeared, Whitney and Cameron (*61*) issued a publication from the United States Bureau of Soils which has attracted more attention than any other single paper dealing with water extracts. In it they gave the amounts of phosphoric acid, nitrates, calcium, and potassium found in the water extracts of both good and poor soils under varying conditions. The extracts employed for these analyses were obtained by stirring 100 gm. of soil in 500 c. c. of distilled water for three minutes. After standing for 20 minutes the liquid was decanted into a cylinder of Brigg's (*6*) filtering apparatus and forced through unglazed Pasteur-Chamberland filters under pressure. They concluded that practically all soils gave essentially identical solutions and that even where only a small quantity of one constituent was present, it was sufficient for the growth of a fair crop if the mechanical condition of the soil was good.

It was concluded also that the soil moisture was practically a saturated solution of the mineral substances present in the soil. Consequently, as fast as salts were removed by the plant further quantities were quickly dissolved, thus keeping the solution at nearly the same concentration throughout the growth of the plant. One of the most significant facts claimed to have been shown by the investigation was that the equilib-

rium of the solution quickly readjusted itself, at least as quickly as the plant disturbed it by withdrawing nutrients.

It was therefore believed that the controlling factors in fertility were moisture and the physical condition of the soil, and not fertilizers or plant nutrients.

This was an entirely new viewpoint in soil investigations and has proved extremely stimulating to other workers, though it should be stated that Cameron (*10–12*) has somewhat modified his conclusions in his later writings.

The first portion of the work of King (*34*), which had been carried on concurrently with that of Whitney and Cameron (*61*) in the Bureau of Soils, appeared a year later. It consisted of three papers, which were published privately by the author, while the remaining three were issued from the Bureau of Soils (*35*).

This work constitutes the most extensive investigation so far carried out on the water extracts of soils. The methods employed were the same as those used in the previous investigation of Whitney and Cameron (*61*). The analyses in the preliminary work were performed on the fresh samples of field soil, but later in the investigation, despite the large error involved, oven-dried samples were employed.

The work of the first season was largely of a preliminary nature and was principally carried out at Goldsboro, N. C. Additional samples were taken in Georgia, Virginia, Maryland, New Jersey, Pennsylvania, and Wisconsin. In the second season the work was more intensive and consisted of a study of eight soil types in the four States of North Carolina, Maryland, Pennsylvania, and Wisconsin. The crops used were cotton, peas, beans, corn, and oats.

Applications of fertilizer consisting of 5, 10, and 15 tons of manure and 300 pounds of guano were made to two crops, corn and potatoes. Analyses of the water extracts of the soils from these varied crops were made from three to six times during the season, as well as numerous extracts of the plant saps.

In general, the results and conclusions drawn were diametrically opposed to those of Whitney and Cameron. Relatively large amounts of nutrients were found to be either actually in solution or in such form that they entered into solution when diluted with distilled water. It was also shown that the application of fertilizers materially increased the amounts of salts recovered from the soils. The largest amounts of salts were, as a whole, found where the yields were largest, and the same results were obtained from the examination of the plant sap.

The influence of farm manures was found to increase not only the crop but also the amounts of soluble salts which could be recovered from the soil.

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At this same period Gola (*18–21*) published the first of his ecological and chemical studies on the relation of the soil solution to the natural plant environment. He attempted to obtain, by a method founded on that of Schlœsing (*46, 47*), an extract which would be similar to the actual soil solution. The soil was broken up so that it would pass through a 2-mm. sieve and was placed in a glass cylinder, 25 cm. high and 4.5 cm. in diameter. A gentle rain of distilled water at the rate of 25 to 30 mm. per square centimeter per 24 hours, was then allowed to fall upon it. After a period of time, which varied with the soil, drops of solution issued from the lower tubulure, and the process was allowed to continue till 25 to 50 c. c. of "pedolytic" solution had been collected. Gola then subjected the saturated soil to pressure and collected what he called the "pedopiezic" solution.

The total solids, and in some cases the total colloids, were determined in these solutions. From the figures so obtained Gola divided up the possible habitats of various plants into some 32 groups which he believed to be controlled by the solution naturally occurring in the soil. In general, he concluded that the relation between the soil and the organs of absorption of the plant was controlled by the osmotic pressure of the soil solution. High concentrations, and, especially, rapid changes in the solution were likely to be harmful to plants, though many may grow successfully in weaker solutions than were normal for them. The principal factor determining the habitat of plants was the concentration of the solution and, to a lesser extent, its chemical composition.

Snyder (*53, 54*), at Minnesota, carried on a short series of investigations in which he studied the absorption of nutrients from soil extracts which were added to sand cultures, believing this condition comparable to the absorption from the soil water. He arrived at conclusions opposed to those of Whitney and Cameron (*61*) and also criticised their results from theoretical considerations.

Gedroitz (*16*), working in Russia, announced that the concentration of the soil solution fluctuated so rapidly that it was impossible for any water extract to give any indication of the character of a soil.

Mitscherlich (*42*) issued an extremely valuable contribution to the study of the water extract. His method of procedure was essentially different from those previously discussed. He employed for the extraction carbon-dioxid-saturated water at 30° C., the maximum temperature which would probably be encountered in the soil. The proportions of soil and water varied from 1 to 5 up to 1 to 30. At least two dilutions were used in each study, generally 1 to 10 and 1 to 25. The soil and water were placed in a thermostat, with a stirring apparatus running into the center of the flask. Carbon dioxid was passed in constantly and the extraction allowed to continue for 11½ hours.

From the data obtained by these varied extractions Mitscherlich graphically estimated the amounts of additional material which were

dissolved by the water extract over and above that portion which was actually in solution. This was a very valuable differentiation. His conclusion that these graphs could be directly produced and would follow the concentration of the actual soil solution is discussed by Hoagland (*27*) in connection with experimental data bearing on this point.

Mitscherlich was able by his procedure to distinguish between fertilized and unfertilized soils and also between various grades of fertilizer application. An excellent feature of his work was the complete and careful estimation of the factor of error involved for all determinations and its influence on the final result.

Ishcherekov (*29*) published the first of numerous attempts, which are still continuing, to obtain the soil solution by the use of various reagents. Briggs (*6*) and Briggs and McCall (*7*) had previously obtained small amounts of solution from the soil by centrifugal force and capillarity when the soil contained moisture slightly in excess of the optimum. Ishcherekov now attempted to obtain the solution from a soil which was close to saturation by pouring a layer of 0.5 cm. of ethyl or methyl alcohol over the soil and assumed that the first portion of clear solution which ran through was the actual soil solution.

The same author later (*30*) reported a series of studies in which he used the methods of the U. S. Bureau of Soils and from which he drew conclusions which were practically in entire agreement with those of Whitney and Cameron (*61*).

Engels (*15*), on the other hand, reported a series of studies in which he used distilled water, carbon-dioxid-saturated distilled water, and 2 per cent citric acid and concluded that the citric acid was the most satisfactory reagent to estimate the soluble material in soil.

Maschhaupt and Sinnige (*41*) conducted an investigation in which carbon-dioxid-saturated water and 2 per cent citric acid were employed and concluded that carbon-dioxid-saturated water was preferable.

Lyon and Bizzell (*40*) have attempted to estimate the density of the soil solution indirectly by determining the relation of the dry matter formed to the transpiration observed. They conclude that the addition of fertilizer caused an increase in the density of the solution and obtained confirmatory evidence by measuring the density of the soil solution with the Wheatstone bridge.

Van Suchtelen (*17*, *59*) has announced a modification of Ishcherekov's (*29*) procedure in which he uses paraffin oil instead of alcohol. He claims to obtain the soil solution in unaltered form, though the full details of his method have not yet appeared. As the first announcement of this method appeared in 1912, and the last statement of its prospective full publication was made by Morgan (*43*) in 1916, it is to be hoped that it may soon appear in its entirety.

Ballenegger (*2*) has used the methods of the Bureau of Soils, as well as determinations of electrical conductivity, in the study of 75 typical Hungarian soils. He concluded that the character of the water solutions may be used to differentiate the various types of soil. The soils investigated varied from the poor gray forest soils to the very fertile "*alfold*" soils.

Toulaikov (*56, 57*) believed, like Gola (*19*), that the osmotic pressure of the soil solution was the important factor in plant growth. He found the optimum to be a pressure of three atmospheres and that the growth of wheat was benefited by an increase up to that point.

Pantanelli (*44*) has attempted to study the concentrations of the solutions of soils from Tripoli by determining the electrical conductivity of the liquids obtained by percolation. He was able by this procedure to differentiate between several classes of cultivated and virgin soils.

Hall, Brenchley, and Underwood (*23*) have reported a noteworthy investigation in which the soils from the Rothamstead experiment plots were used. Solutions were prepared using 20 kgm. of soil and 35 kgm. of water, and wheat and barley plants were grown therein. From the analysis of these solutions and the growth of the plants in them it was concluded that—

> The composition of the natural soil solution as regards phosphoric acid and potash is not constant, but varies significantly in accord with the composition of the soil and its past manurial history. Within wide limits the rate of growth of a plant varies with the concentration of the nutritive solution irrespective of the total amount of plant food available. When other conditions such as the supply of nitrogen, water and air are equal, the growth of crops will be determined by the concentration of the soil solution in phosphoric acid and potash; which, in its turn, is determined by the amount of these substances in the soil, their state of combination and the fertilizer applied.

These authors did not find any toxic effect on soils which had grown wheat and barley for even 60 years. Growth in the soil solutions agreed with the growth in culture solutions containing equivalent amounts of phosphoric acid and potash.

In a series of nutritive solutions of various degrees of dilution the growth varied directly, but not proportionately, with the concentration of the solution.

Finally, the authors concluded that the results of the investigation restored the earlier theory of the direct nutrition of plants by means of fertilizers and nullified the theories advanced by Whitney and Cameron (*61*).

Bouyoucos and McCool (*5*) have proposed an ingenious method for determining the concentration of the soil solution directly by means of the freezing point. Use has been made of this procedure in the present investigation by Hoagland, and its application and limitation are discussed in a separate paper (*27*).

Bogue (*3*) has published a brief investigation in which he leached four soils with water and also studied their absorptive capacity for potassium and phosphates. From his work he agreed with Whitney and Cameron that the composition of the soil moisture is not influenced by the chemical composition of the soil, but instead is dependent on the mechanical texture of the individual soil.

Jensen (*32*) reported a series of observations on eight sugar-beet plots at Rocky Ford, Colo. The methods of investigation employed were those of the U. S. Bureau of Soils (*50*). The plots receiving composted manure showed nearly twice as much water-soluble potash in the surface foot as did any of the other plots. The seasonal averages of this element were not appreciably influenced by any other fertilizer treatment. There was a decrease noted in the quantity of water-soluble potash from the middle of May till the middle of July. After that time the quantity increased to approximately the amount that had been found earlier in the season. None of the treatments resulted in a marked increase of soluble phosphates, and the variation in this compound was less than in any other element measured.

Jordan (*33*) has recently published the results of an investigation in which nine soils were analyzed by complete analysis, acid extraction with hydrochloric acid of 1.115 specific gravity, 10 days' leaching with water, *N/200* and *N/25* hydrochloric acid, and five hours' extraction with the last three solutions. Vegetation experiments were conducted with the soils in the greenhouse during two years. At the close of this time it was impossible to establish any relationship between any of the elements determined and the crop-producing capacity. There appeared to be a slight relationship between the total soluble matter in the soil and crop production, but it was not consistent in all cases. The final conclusion was that no method had been developed by which the fertility of a soil could be measured through laboratory investigation.

Harris and Butt (*24*), working in Utah, have studied the effect of varying amounts of irrigation water upon the development of nitrates and soluble salts in cropped and fallow soils. They found notable differences between the cropped soil and the fallow duplicate, but did not find that these differences were related to variation in the crop yield.

SCOPE OF THE INVESTIGATION

The foregoing discussion indicates the contradictory nature of the results already obtained. Two of these moot points especially open to further study are:

1. The relationship between the soluble soil nutrients in cropped and uncropped soils.

2. The relationship between the soil extract and the crop produced thereon.

These questions are the subject of the present study.

METHOD OF ATTACK

It is evident that in any soil a large number of complex factors determine production. These factors, besides influencing the final crop obtained, can also conceivably modify the condition of the plant food in the soil itself. It becomes very clear then that a chemical study of the soil solution can hope to obtain a fair degree of success, only if a number of these modifying factors are subordinated or made comparable.

Prominent among such factors are the influence of physical texture, climate, and moisture. To reduce the effect of physical texture, two types of soils were chosen: silty clay loams and fine sandy loams. The representatives of each class were selected with as uniform a physical texture as possible. Comparisons can therefore be made between the soils within each class, with the assurance that the physical factor is reduced to the minimum.

The effect of climate was made uniform by transporting exceptionally large samples of surface soil to Berkeley. The samples were then sifted and placed in containers which will be described later. Moisture conditions were made as uniform as possible by adding just sufficient distilled water to keep the soils at their optimum content.

SELECTION OF THE SOILS

The soils used in the investigation were chosen according to the mapping of the Bureau of Soils of the United States Department of Agriculture. The silty clay loams were all chosen from the Yolo series. Of these, three were taken from the Sacramento Valley and three from the Santa Clara Valley. The past history of all these soils was very different, as shown in Table I. The crops grown on them were equally divided between orchard crops (prune, almond, peach) and field crops. None of the orchards had received any special treatment. Among the field soils, the sample from the University Farm at Davis was the only one which had been manured, though its cropping was no more varied than No. 4 from the Santa Clara Valley. Soil 3 from Yolo had a less varied history than any other in either set. This soil had been under cultivation since about 1860, and, except for two years in sugar beets, 1911 and 1912, it had been steadily cropped with wheat or barley each year. Its past production was stated to be good, fully the average for that section, though no exact record had been kept of the yields.

TABLE I.—*Classification and history of the soils used in this investigation*

Soil No.	Soil series and type.	Origin.	Crop grown.	Past treatment.
1	Yolo silty clay loam.	Sacramento Valley (University Farm, Davis).	Field crops...	Early planting of grain; 1909–1911, barley; 1912, fallow and manure; 1913–14, barley.
2	.....do..........	Sacramento Valley (Yolo).	Almond orchard.	Formerly grain; almond orchard 12 years old.
3	.....do..........	.....do..........	Barley.......	Planted about 1860; since then barley and wheat, except sugar beets in 1911–12.
4	.....do..........	Santa Clara Valley (San Jose).	Field crops..	Originally grain; later orchard; several years alfalfa; three years field crops.
5	.....do..........	.....do..........	Prunes.......	Originally grain; prune orchard about 20 years old.
6	Yolo clay loam...	Santa Clara Valley (Lawrence).	Peaches......	Originally grain; peaches for 8 years; heavy crop, about 12 tons per acre.
7	Hanford fine sandy loam.	Southern California (Arlington).	Oats........	Originally grain; about 1890 put into alfalfa for 13 years; potatoes 2 years, alfalfa 4 years, oats 5 years; yield of oats, 4 tons of hay per acre.
8	Fresno fine sandy loam.	San Joaquin Valley (Fresno).	Seedless grapes.	Originally grain; 14 years in Sultanina (Thompson Seedless) grapes. Production about 2 tons of raisins per acre for last 6 years.
9	Kimball fine sandy loam.	Southern California (Redlands).	Navel oranges.	25 years in oranges; previously bare land. A great variety of fertilizers had been used.
10	Tejunga fine sandy loam.	Southern California (San Fernando Valley).	Peaches......	Originally 15 years in prunes; now 10 years in peaches; small amount or manure the only treatment.
11	Madera fine sandy loam.	San Joaquin Valley (Kearney Park).	Navel oranges.	Orange trees about 15 years old.
12	Arnold fine sandy loam.	San Joaquin Valley (foothills).	Oats........	In cultivation about 40 years; early crops largely wheat; last four or five years biennial crops of oats; alternate year summer fallow.
13	Unnamed fine sandy loam.	Mendocino County coast.	Virgin........	Very shallow soil, about 1 foot in depth underlain by clay subsoil.
14	Standish fine sandy loam.	Honey Lake region.	.....do.......	Desert soil, small shrubs and weeds, natural growth.

The fine sandy loams had an even more varied past history. Three of these soils were from southern California. One of these was from a Redlands orange grove which had been treated in the past with a great variety of fertilizers. A second was from a peach orchard of the San Fernando Valley, and the third from the Riverside area had been devoted to field crops.

Three fine sandy loams were obtained in the San Joaquin Valley. Two from the vicinity of Kearney Park, in oranges and Sultanina (Thompson Seedless) grapes, had an excellent record for past production. The third from the foothills near Oakdale was purposely chosen from a body of Arnold fine sandy loam where the growth of oats was unusually small, though the soil was normal in depth and drainage. The last two fine sandy loams were virgin soils. One of these (No. 13) came from the coast of Mendocino County, and was an extremely poor, shallow soil. In fact, it later developed that this soil was very acid; therefore no deductions have been drawn from the results obtained with it. The other virgin sample was obtained from the Honey Lake area. This soil was considered to be rather in the class of desert soil, but was not especially deficient in organic matter.

The soils of both types were selected to include as many past treatments and crops as possible. They had, of course, been exposed to very different climatic influences. This is an unavoidable factor in California, with its great diversity of local conditions. It is believed that these various influences have been largely neutralized by the cropping of the first season, 1915, under uniform conditions. Greater emphasis is therefore attached to the results of the past year (1916).

The attempt was also made to choose soils which, though of uniform physical texture, would have a very different crop-producing power. In this way it was hoped that some light might be thrown on the relation between yield and water-soluble nutrients.

In Table II are given the moisture equivalents, hygroscopic coefficients, specific heat, and mechanical analyses of the 14 soils. Moisture equivalents are obtained by the method of Briggs and Shantz (*8*); hygroscopic coefficients by the method of Hilgard (*26*). Specific heat was determined by heating 20 gm. of soil in a tin-foil container until it reached a temperature of approximately 100° C. It was then lowered into an insulated tank of water of known temperature and volume. The amount of heat contained in the soil was measured by the increase of temperature of the water. The mechanical analyses were performed by the method of the Bureau of Soils (*9*). It should be noted that the analyses as reported were performed on the same samples which were used for the chemical analyses. These samples had been passed through a 1-mm. sieve. This was done in order that the chemical and physical analyses should be made on identical portions. The percentage of gravel by this screening process is also stated, so that the mechanical analyses

may, if desired, be recalculated to the usual form. A study of these figures shows that the members of each group of soils in physical texture are as similar as was desired. From these analyses Mr. C. F. Shaw, of this Station, states that the silty clay loams would be accurately classed as—

> clay loams with a very high percentage of fine sand and silt, and that with the exception of No. 6 they would function as very uniform silty clay loams. No. 6 would be classed as a clay.

The fine sandy loams are considered to be quite uniform in texture, so far as porosity, ease of root penetration, and behavior under cultivation are concerned. The moisture equivalents are very different, so that at first glance it would appear that the plants would be unequally supplied, especially in the fine sandy loams. It will be pointed out later, however, that this view does not consider the fixed or "unfree" water of the soil. The amount of moisture, therefore, which is available to the plants, as shown by the wilting coefficient, and also the amount which affects the concentration of the soil solution, can not be judged from the moisture equivalent alone.

TABLE II.—*Moisture equivalents, hygroscopic coefficients, specific heat, and mechanical analysis of experimental soils*

Test No.														
Soil	1	2	3	4	5	6	7	8	9	10	11	12	13	14
MOISTURE EQUIVALENTS														
Test 1	27.51	25.23	27.44	23.62	24.12	30.57	13.61	7.77	9.95	18.41	16.29	9.15	18.57	17.84
Test 2	27.27	25.06	27.10	23.95	23.29	30.09	13.45	7.94	9.27	17.98	16.00	10.00	19.02	17.45
Test 3	27.08	24.93	27.26	24.53	24.38	30.24	13.06	8.24	9.73	18.76	16.61	9.72	18.22	17.99
Test 4	27.21	24.77	27.69	24.01	23.75	30.22	13.10	8.30	10.53	......	16.24	10.01	18.43	......
Average	27.28	25.00	27.37	24.03	23.88	30.28	13.31	8.06	9.87	18.38	16.28	9.72	18.56	17.99
HYGROSCOPIC COEFFICIENTS														
Test 1	7.95	6.09	8.33	5.49	8.11	8.82	2.83	1.15	1.57	2.93	3.14	1.85	1.31	4.72
Test 2	7.92	6.36	8.08	5.77	8.14	8.84	2.94	1.20	1.49	2.95	3.18	1.68	1.30	4.76
Average	7.94	6.23	8.20	5.63	8.12	8.83	2.89	1.17	1.53	2.94	3.16	1.77	1.31	4.74
SPECIFIC HEAT														
Test 1	0.2122	0.2057	0.193	0.1901	0.2246	0.243	0.1969	0.1758	0.230	0.184	0.2480	0.1807	0.1933	0.224
Test 2	.2095	.2042	.200	.2008	.2245	.242	.1876	.1661	.234	.188	.2501	.1656	.1860	.230
Average	.211	.205	.1965	.1954	.2245	.2425	.1918	.1709	.232	.186	.2490	.1731	.1897	.227

TABLE II.—*Moisture equivalents, hygroscopic coefficients, specific heat, and mechanical analysis of experimental soils*—Continued

MECHANICAL ANALYSES

[Averages of two analyses]

Kind of soil.														
Soil	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Fine gravel (1–2 mm.).	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.016	0.00	0.00	0.00	0.04	0.00
Coarse sand (1–1.5 mm.)	.15	.60	.60	2.09	1.84	.746	2.82	1.95	9.986	1.57	2.77	4.99	7.77	3.09
Medium sand (0.5–0.25 mm.).	.23	1.50	1.04	1.24	1.70	.574	2.87	3.19	6.308	2.94	2.818	4.38	14.55	4.05
Fine sand (0.25–0.010 mm.)	1.69	8.00	3.53	5.47	9.61	1.786	15.60	21.22	21.730	16.62	20.945	23.27	13.49	24.54
Very fine sand (0.01–0.05 mm.).	32.62	31.26	26.28	30.32	32.73	21.775	53.67	50.63	43.67	38.76	39.34	46.24	15.70	38.94
Silt (0.05–0.005 mm.)	42.68	36.97	38.12	37.99	30.52	37.415	16.01	17.57	10.87	26.25	19.075	9.61	34.72	15.55
Clay (0.005–0 mm.)	24.17	22.47	28.47	25.08	24.72	39.380	9.77	5.56	8.19	13.37	14.62	9.44	12.95	13.87
Sum of percentages	101.54	100.80	98.68	102.19	101.12	101.676	100.58	100.12	100.77	99.51	99.568	97.93	99.22	100.04
Gravel removed by previous screening	.11	.09	.39	6.15	3.38	.11	3.64	.99	6.41	.59	2.12	1.57	1.15	2.21

In choosing the soils in the field the following plan was adopted. One soil of each type was taken as the typical sample. Then each succeeding member of that group was chosen by comparison with a small sample of this type. This same small sample was carried while all the work was being done on that group, and constant comparisons were made when there was ground for doubt. In this way it was believed that each member of a group would approach very closely to the other members in general physical texture.

The samples in each case were taken from the top foot of soil. Preliminary borings with a soil auger were made in order to ascertain whether the subsoil was free from hardpan or other abnormal factors which might influence the surface soil. The amount of each of these samples was 2½ tons. The soil was shipped in new, heavy burlap sacks holding approximately 100 pounds each. Every attempt was made to prevent undue drying or exposure to sunshine after the soil was sacked.

Upon the arrival of the samples in Berkeley they were immediately passed through ¼-inch concrete sieves to obtain a uniform physical texture. This work was performed with all possible expedition, and it is believed that they had a normal bacterial flora when placed in the containers. None of the soils, except No. 14, was actually air-dry when finally prepared for use. The ammonifying and nitrifying powers of the soils have been determined as a test of normal biological activity. This work was performed at the beginning and close of the season of 1915 and at the end of the growing season in 1916. The tests were carried out

by the well-known tumbler method. All the soils showed a satisfactory capacity for ammonification.

At the close of the season of 1915, soils 12 and 14 were noticeably lower in nitrifying power than the others of the group. At the close of 1916, soil 12 alone showed a significant lower range in nitrification. With these exceptions the soils gave results which indicated normally active bacterial floras. The detailed data are not considered sufficiently illuminating to justify their inclusion in this paper.

SOIL CONTAINERS AND INSTALLATION

The soil containers selected were 30 inches wide, 60 inches long, and 18 inches deep. They were made of No. 24 galvanized iron and were thoroughly coated with asphaltum varnish. When filled, each contained approximately 1,700 pounds of soil. The design of the containers

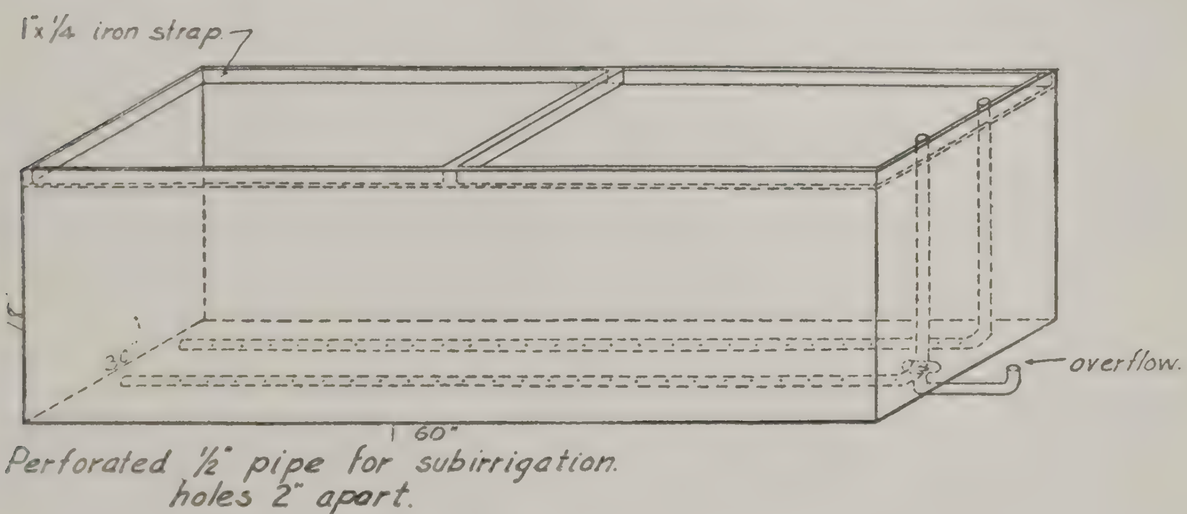

FIG. 1.—Design of soil containers.

is shown in figure 1. It will be observed than an outlet is placed in the bottom at one end. This outlet not only prevented the accumulation of excess water, but also gave additional aeration to the lower soil. The escape pipes may possibly be seen in the illustration of the wire house inclosing the containers (Pl. 14, A).

Distilled water was the only moisture used at any time. This was added partly between the rows of grain by a long-spouted sprinkling can and partly by subirrigation through the two perforated pipes running from end to end of the containers. So far as possible, only enough water was added at one application to keep the soil at optimum moisture. It was found possible to observe the moisture condition quite closely by drawing out a core of soil with an 18-by-¾-inch cheese trier, and then replacing the soil.

The containers numbered 28, 2 for each soil, and were arranged on level mud sills in two duplicate sets, as indicated in the accompanying diagram (fig. 2). By the above arrangement the smallest possible

external surface of each container was exposed to any temperature change. These changes were further minimized by surrounding the set of containers with a boxing, 18 inches high, placed 6 inches from the outside of the entire group. This 6-inch space was then filled compactly with local soil. The insulation furnished by this arrangement was excellent. No difference in growth was observed in any portion of the containers.

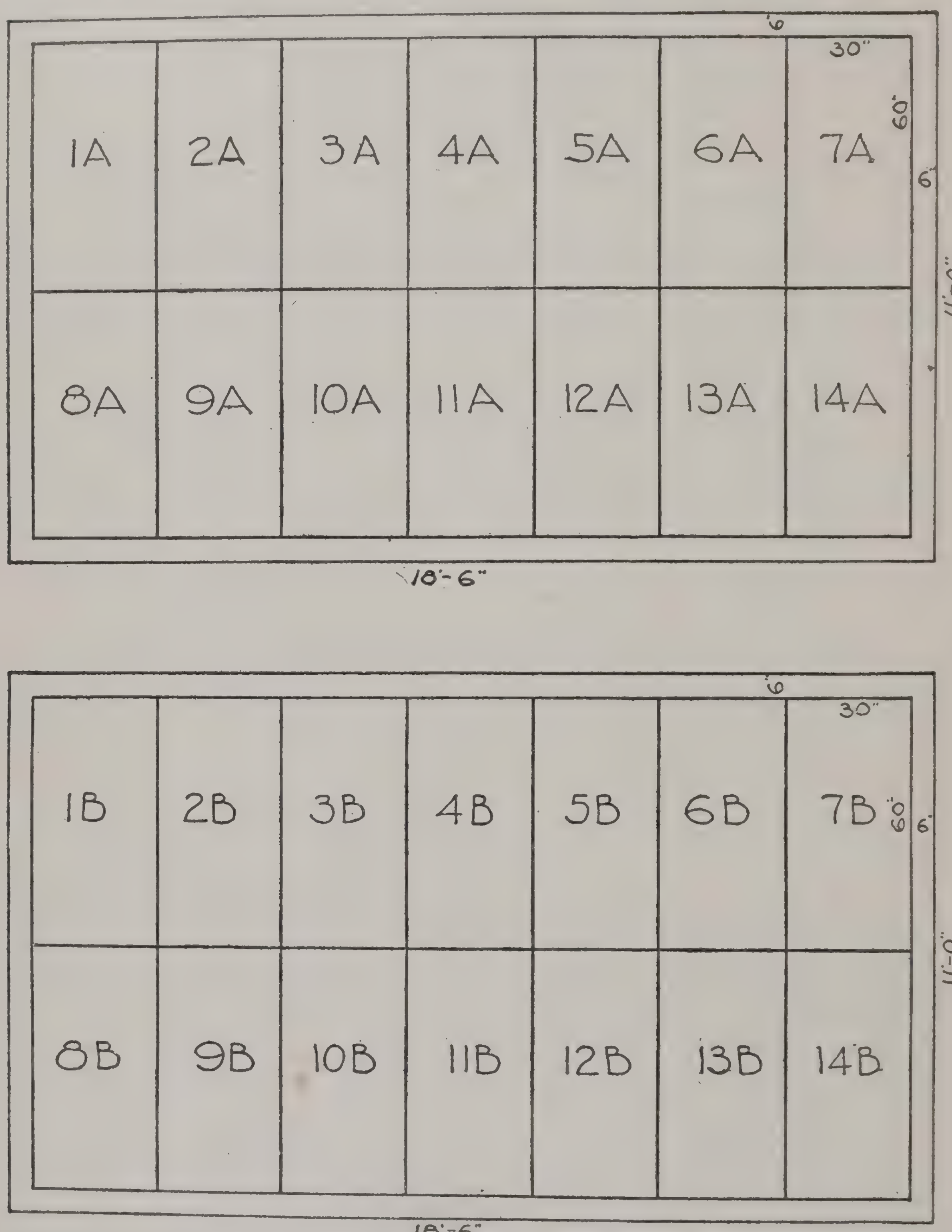

FIG. 2.—Diagram of the arrangement of the soil containers.

Protection from birds was assured by a framework, 4½ feet in height above the containers, covered with 1-inch-mesh wire netting. The general arrangement of the two sets is shown in Plate 14, A.

During the growing season selected for these experiments, from May to September (the first season, however, being from June to October), very little rain falls in Berkeley. To protect the soils from flooding by any heavy showers that might occur, waterproof canvas covers have been provided and have been put over the wire houses in a few emergencies. During the season of heaviest rains these covers are kept on, and the containers are never flooded or subjected to leaching by rain. In this season between crops the soils are watered with distilled water at intervals, so that they are close to the optimum at all times. It is, of course, true that this is not comparable to the seasonal changes to which field soils are subjected. On the other hand, such seasonal changes of rainfall and drouth are never comparable for any two seasons or any two places. Many orchard soils in this State, by constant cultivation and irrigation in the summer, retain a very regular moisture supply in the soil. The moisture conditions of this experiment are, in fact, very similar to those found in such cases. The orchard, in addition, is subjected to occasional large excesses of water from rainfall.

After filling the containers with the prepared soils, the excess portions of sifted soil were stored in the set of tightly covered bins shown in Plate 14, B. This soil was available for supplying the small portions removed by sampling and also furnished a stock for additional studies.

ANALYTICAL PROCEDURE

In the season of 1915 the analytical methods used were those outlined in United States Department of Agriculture Bureau of Soils Bulletin 31 (*50*). With pure solutions containing only a single compound the determinations obtained by these methods are very satisfactory. Before the close of the season considerable doubt was felt in regard to certain of the results obtained. This applied especially to calcium, potash, and phosphate. Before entering upon the work of the season of 1916 a series of studies were outlined to test the accuracy of the methods which had been in use. The preliminary work previously performed by the procedures in Bulletin 31 had all been carried out with solutions which contained only one pure salt of the radical to be determined. Under these conditions the results were extremely accurate.

In the studies now undertaken three sets of solutions were prepared to simulate as closely as possible the highest, average, and lowest concentrations ordinarily obtained in soil extracts. The salts used were monocalcium phosphate, calcium nitrate, magnesium sulphate, potassium chlorid, and ammonium chlorid. The solutions were made up with distilled water which had stood in ordinary glass bottles and so contained small amounts of sodium and silica, though not of course comparable to the amounts which occur in water extracts from soils.

Quadruplicate portions of these solutions were analyzed without further treatment. Large aliquots from the same stock bottles were also passed through new and old Pasteur-Chamberland filters, and some portions were treated with G. Elf carbon black. These treatments were included to check the possible effect of absorption of elements by the filter candles, as well as the effect of the carbon black which was used to decolorize soil extracts containing organic matter.

Table III gives the results of the analyses of the untreated and of the filtered solutions. It will be seen that the determinations of ammonia and nitrate are satisfactory throughout. The methods used are essentially those almost universally applied in sanitary water analysis (*1*) and with the proper precautions are not believed to be open to criticism. The results for potassium, calcium, sulphate, and phosphate are seen to be extremely inaccurate in the lowest concentration. Of these the calcium is by far the most questionable. The error in the determination of this element is so large that the results for calcium obtained in 1915 have consequently been discarded. Even with the potash and phosphate the percentage of error is very large in some determinations. In the higher range of concentrations the results are much closer throughout, and if it were possible to confine all the work done to such solutions there would perhaps be no objection to the methods employed. In many solutions smaller concentrations of one or two elements are frequently encountered. In such a case the error may be 50 per cent or more, either plus or minus. Such a variation can readily obscure any significant change in concentration. It became necessary to obtain a more reliable procedure for potassium, calcium, and phosphate, all of which are extremely important elements in any study of soluble plant nutrients.

TABLE III.—*Analysis (in parts per million) of dilute, average, and strong solutions by the methods outlined in United States Department of Agriculture Bureau of Soils Bulletin 31*

Solution and treatment.	NH_3		NO_3.		K.		Ca (turbidimetric).		Mg (colorimetric).		SO_4 (turbidimetric).		PO_4 (colorimetric).	
	Theory.	Determined.	Theory.	Determined.	Theory.	Determined.	Theory.	Determined.	Theory.	Determined.	Theory.	Determined.	Theory.	Determined.
Dilute, untreated	0.50	0.48	4.00	4.08	1.2	5.72	1.5	1.76	0.50	0.51	1.99	2.72	1.0	2.5
	.50	.48	4.00	4.08	1.2	1.92	1.5	1.68	.50	.46	1.99	2.68	1.0	2.4
	.50	.48	4.00	4.00	1.2	1.92	1.5	3.80	.50	.49	1.99	3.60	1.0	1.6
	.50	.48	4.00	4.16	1.2	1.80	1.5	2.08	.50	.44	1.99	2.60	1.0	1.9
Dilute, filtered through Pasteur filters	.50	.45	4.00	4.16	1.2	3.40	1.5	.48	.50	.44	1.99	2.68	1.0	1.6
	.50	.45	4.00	4.08	1.2	3.60	1.5	1.84	.50	.44	1.99	2.60	1.0	1.7
	.50	.40	4.00	4.08	1.2	3.20	1.5	1.24	.50	.46	1.99	2.76	1.0	1.9
	.50	.45	4.00	4.08	1.2	3.12	1.5	1.60	.50	.44	1.99	2.72	1.0	1.7
Dilute, treated with carbon black and filtered through Pasteur filters	.50	.45	4.00	3.92	1.2	2.12	1.5	1.44	.50	.56	1.99	2.88	1.0	1.7
	.50	.40	4.00	3.92	1.2	2.72	1.5	1.28	.50	.56	1.99	2.80	1.0	1.6
Dilute, passed through new Pasteur candles	.50	.42	4.00	3.68	1.2	3.24	1.5	1.92	.50	.54	1.99	2.60	1.0	1.5
	.50	.40	4.00	3.84	1.2	2.64	1.5	1.08	.50	.49	1.99	3.68	1.0	1.7
Average, untreated solution	1.50	1.66	20.00	19.2	6.00	4.30	7.5	8.00	2.50	2.36	9.8	9.00	5.0	6.0
	1.50	1.58	20.00	19.6	6.00	5.10	7.5	7.70	2.50	2.41	9.8	8.8	5.0	5.2
	1.50	1.58	20.00	20.0	6.00	5.88	7.5	7.50	2.50	2.30	9.8	10.0	5.0	5.2
	1.50	1.58	20.00	20.0	6.00	6.24	7.5	7.90	2.50	2.36	9.8	8.8	5.0	5.0
Average solution filtered through Pasteur filters	1.50	1.61	20.00	19.2	6.00	6.64	7.5	7.90	2.50	2.66	9.8	9.6	5.0	4.8
	1.50	1.61	20.00	18.8	6.00	8.69	7.5	8.00	2.50	2.61	9.8	9.2	5.0	4.0
	1.50	1.61	20.00	19.2	6.00	8.40	7.5	7.60	2.50	2.61	9.8	8.8	5.0	4.6
	1.50	1.64	20.00	18.8	6.00	8.00	7.5	7.70	2.50	2.66	9.8	9.2	5.0	5.0
Average solution treated with carbon black and filtered through Pasteur filters	1.50	1.64	20.00	18.6	6.00	6.00	7.5	8.00	2.50	2.61	9.8	13.4	5.0	4.3
	1.50	1.64	20.00	18.6	6.00	6.00	7.5	8.20	2.50	2.43	9.8	11.8	5.0	4.6
Average solution passed through new Pasteur filters	1.50	1.64	20.00	19.2	6.00	6.96	7.5	8.30	2.50	2.30	9.8	11.8	5.0	4.8
	1.50	1.64	20.00	18.0	6.00	6.56	7.5	7.80	2.50	2.41	9.8	11.8	5.0	4.7
Strong solution, untreated	3.00	3.07	38.00	37.6	12.00	10.66	15.0	16.60	5.00	5.31	19.7	20.0	10.0	10.4
	3.00	2.94	38.00	37.6	12.00	10.66	15.0	16.40	5.00	4.80	19.7	19.0	10.0	10.8
	3.00	2.94	38.00	38.4	12.00	13.12	15.0	16.40	5.00	4.74	19.7	18.2	10.0	10.4
	3.00	2.94	38.00	38.4	12.00	11.76	15.0	17.00	5.00	4.93	19.7	19.0	10.0	10.2
Strong solution passed through Pasteur candles	3.00	2.86	38.00	38.4	12.00	12.32	15.0	16.60	5.00	4.93	19.7	19.5	10.0	9.8
	3.00	3.07	38.00	37.6	12.00	12.49	15.0	16.80	5.00	4.80	19.7	18.2	10.0	9.2
	3.00	2.90	38.00	37.6	12.00	13.56	15.0	16.40	5.00	4.86	19.7	18.5	10.0	9.2
	3.00	2.94	38.00	38.4	12.00	11.60	15.0	16.00	5.00	4.74	19.7	18.7	10.0	9.4

The determination of magnesium, though it is the same procedure as used for phosphate, has, at least in this series, given more accurate results. The same standard solution is used in this method as in the phosphate determination, and has a value approximately one-fourth as great when expressed in terms of magnesium, a fact which would decidedly tend to reduce the percentage of error in the final result. The same colorimetric procedure for magnesium is still in use, as no more satisfactory method for small amounts of this element has so far been developed. The sulphate determination is seen to be more accurate than that of calcium, but the error in low concentrations is from 35 to 85 per cent. This procedure has therefore been dropped and the sulphate determinations are not included in the 1915 charts.

In planning the work for 1916 it was evident that a smaller number of more accurate determinations were to be preferred to a larger number on which little reliance could be placed. With this idea in mind, work

was undertaken in which larger aliquots than those recommended in Bulletin 31 were used. The results obtained by the use of larger portions of solution yielded, in general, more reliable figures. If larger quantities of solution were to be used, a great deal of the argument in favor of the colorimetric and turbidimetric procedures disappeared. The small portions of solution required for these methods have always been considered one of their great merits. In the soil extracts which contained the largest quantities of plant nutrients, if larger aliquots were taken, it was very clear that the ordinary volumetric and gravimetric procedures could be used. It then became important to learn whether the standard methods of determination could not be applied to the weaker solutions if a carefully standardized technic were adopted. In the work undertaken along this line the dilute and average solutions previously mentioned were used. As a result, it is believed that a more satisfactory procedure than the colorimetric has been developed for phosphates, calcium, and potash. The methods used are quite well known, but a great deal of work was done to establish the exact conditions for accurate results with the dilute solutions employed.

The description of the detailed procedures used follows.

PHOSPHATE.—Evaporate two portions of soil extract of 200 c. c. each in a 200-c. c. porcelain casserole. This size of casserole stands ignition excellently. Add a few drops of hydrochloric acid diluted 1 to 1 before the above solution reaches dryness to aid in decomposing soluble silicates. Ignite the dry residues in the casserole over a Meeker burner at a moderate temperature till a grayish white residue is obtained. Cool. Take up the residue with 10 c. c. of nitric acid diluted 1 to 9. Cover with a watch glass and digest on the steam bath for approximately 10 minutes to insure complete solution. Filter into a 200-c. c. Erlenmeyer flask. Wash the casserole with two more portions of 10 c. c. each of hot nitric acid diluted 1 to 9. Wash the dish, and filter with small portions of hot water. Keep the total volume of solution to about 50 c. c. Cool. Add a few drops of methyl orange indicator and neutralize rapidly with concentrated ammonia. Bring just to acid reaction with concentrated nitric acid. Add 2 c. c. of saturated ammonium nitrate. Place the flasks and a quantity of properly acidified ammonium-molybdate solution [1] in a water bath at 50° C. When all the solutions have reached this temperature, add 5 c. c. of molybdate to each determination. Keep at 50° C. for ½ hour. Remove from the water bath, and filter at once on prepared asbestos felts. Wash with cold distilled water till free from acid by the usual tests.[1] Transfer filter felt to the same flask, using approximately 25 c. c. of distilled water free from carbon dioxid. Add 15 c. c. of sodium hydroxid of which 1 c. c. is equivalent to 0.1 mgm. of phosphorus-pentoxid and observe carefully whether the solution of the yellow precipitate is complete. Titrate the excess alkali with hydrochloric acid of the same strength, using phenolphthalein as indicator. Calculate results to either elementary phosphorus or the phosphation, as desired.

CALCIUM.—Evaporate 200 c. c. of water extract to dryness in a 200-c. c. casserole. Ignite at a moderate temperature over a Meeker burner till a grayish white ash is obtained. This step is desirable to remove traces of organic matter, even though the

[1] WILEY, H. W., ed. OFFICIAL AND PROVISIONAL METHODS OF ANALYSIS, ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS, AS COMPILED BY THE COMMITTEE ON REVISION OF METHODS. U. S. Dept. Agr. Bur. Chem. Bul. 107 (rev.), p. 4. 1908. Reprinted in 1912.

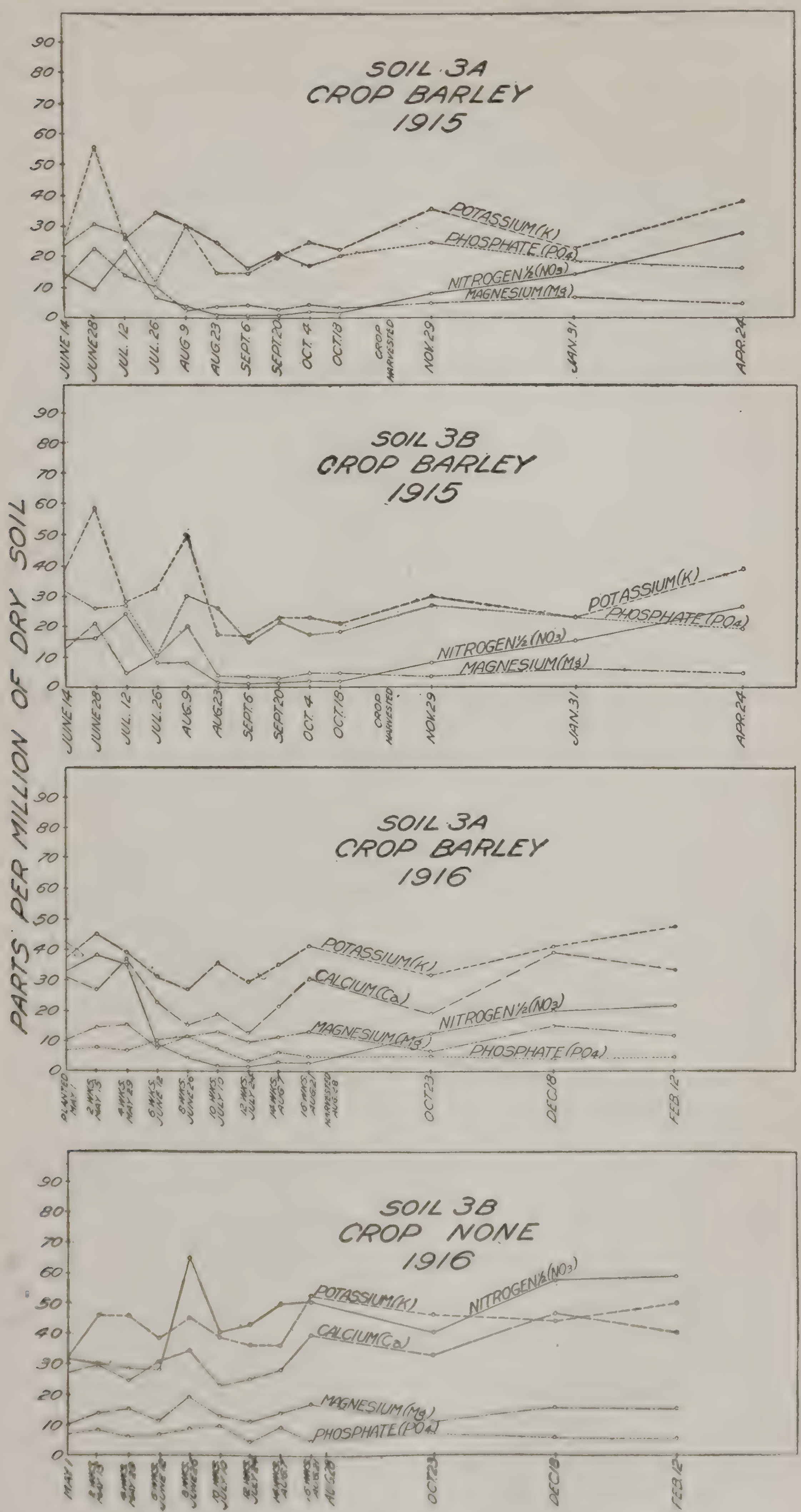

FIG. 10.—Graphs of the seasonal studies of the water extract of soil 3, Yolo silty clay loam.

27809°—18——4

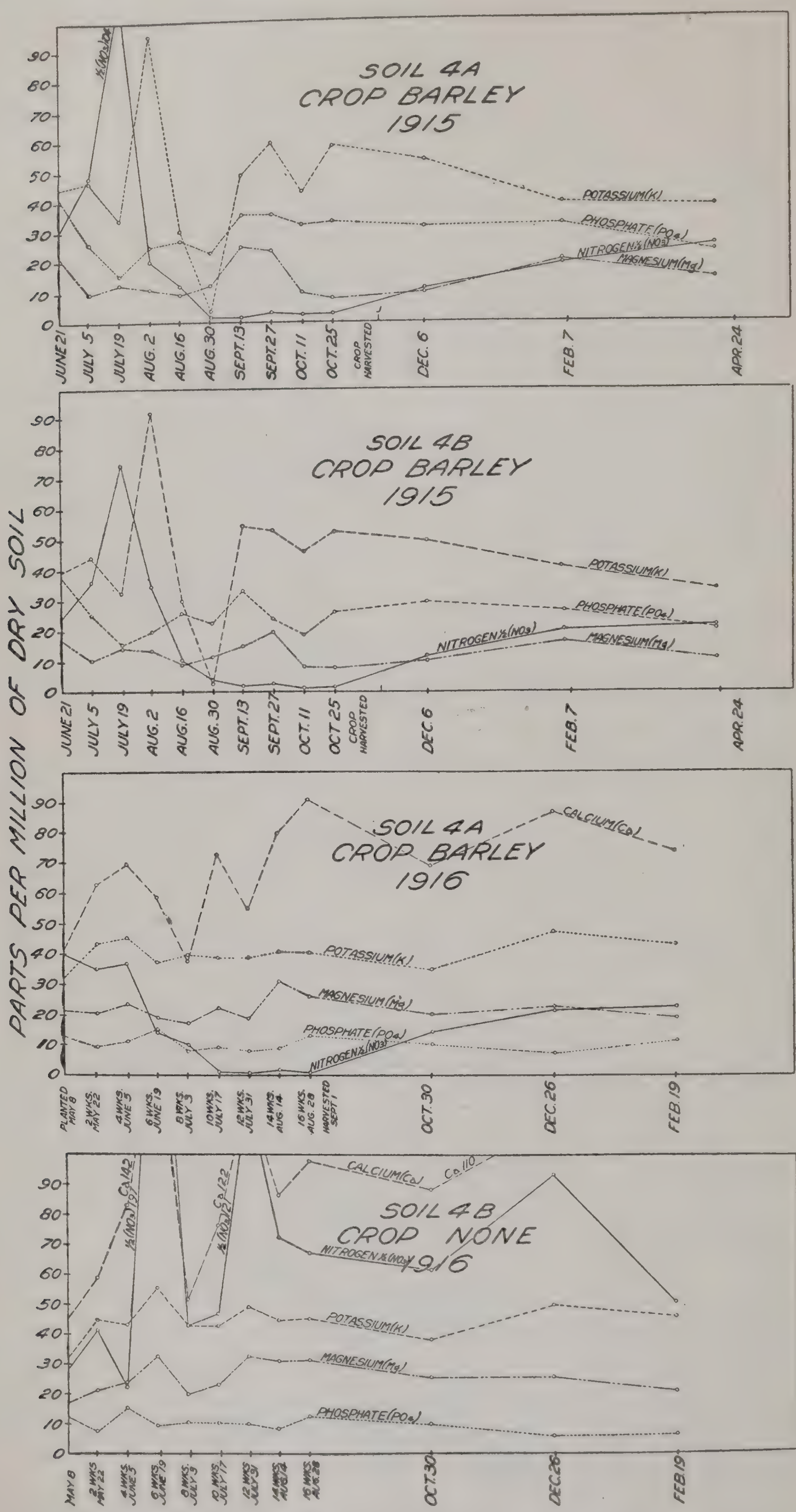

Fig. 11.—Graphs of the seasonal studies of the water extract o fsoil 4, Yo'o silty clay loam.

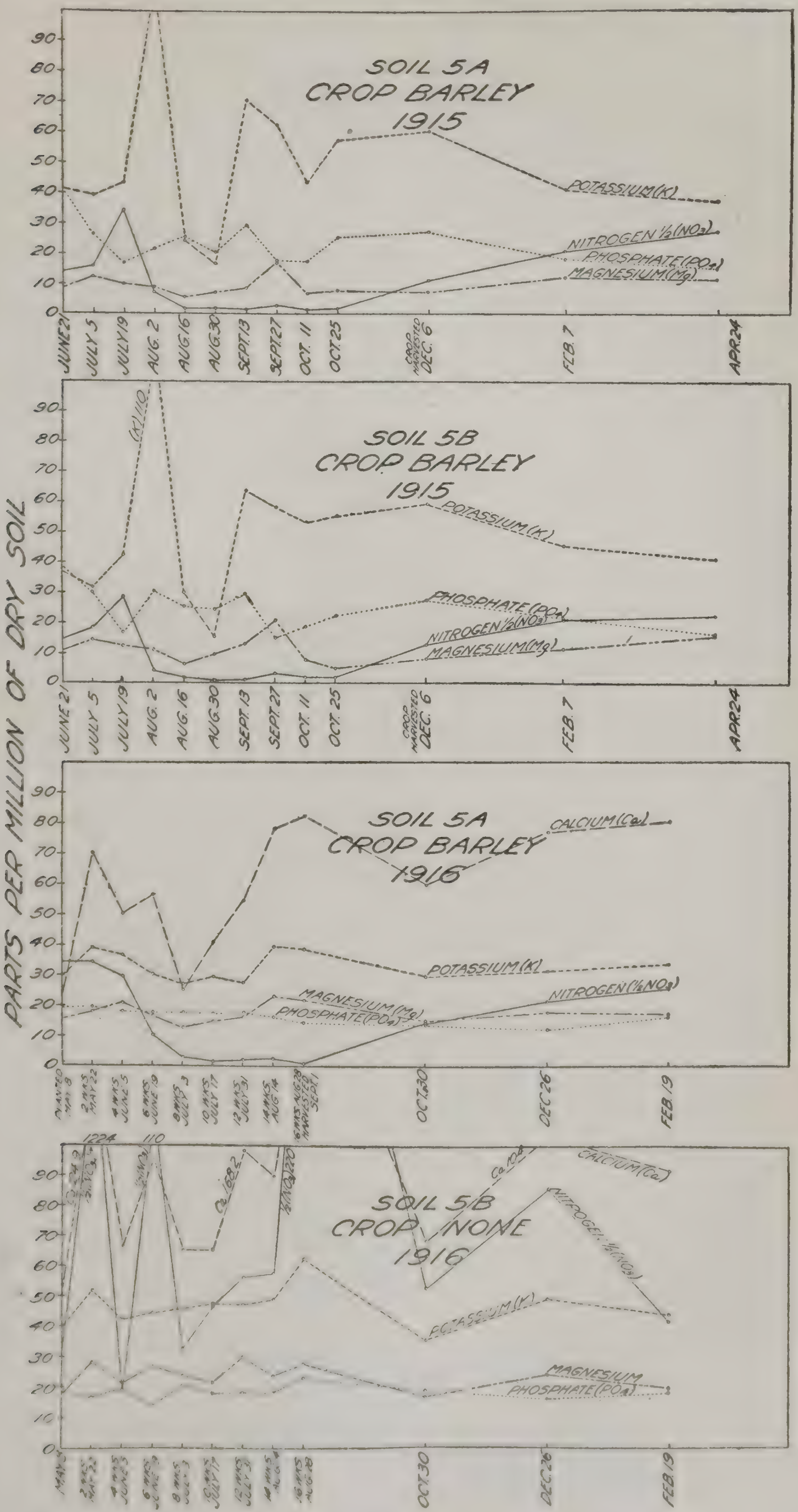

FIG. 12.—Graphs of the seasonal studies of the water extract of soil 5, Yolo silty clay loam.

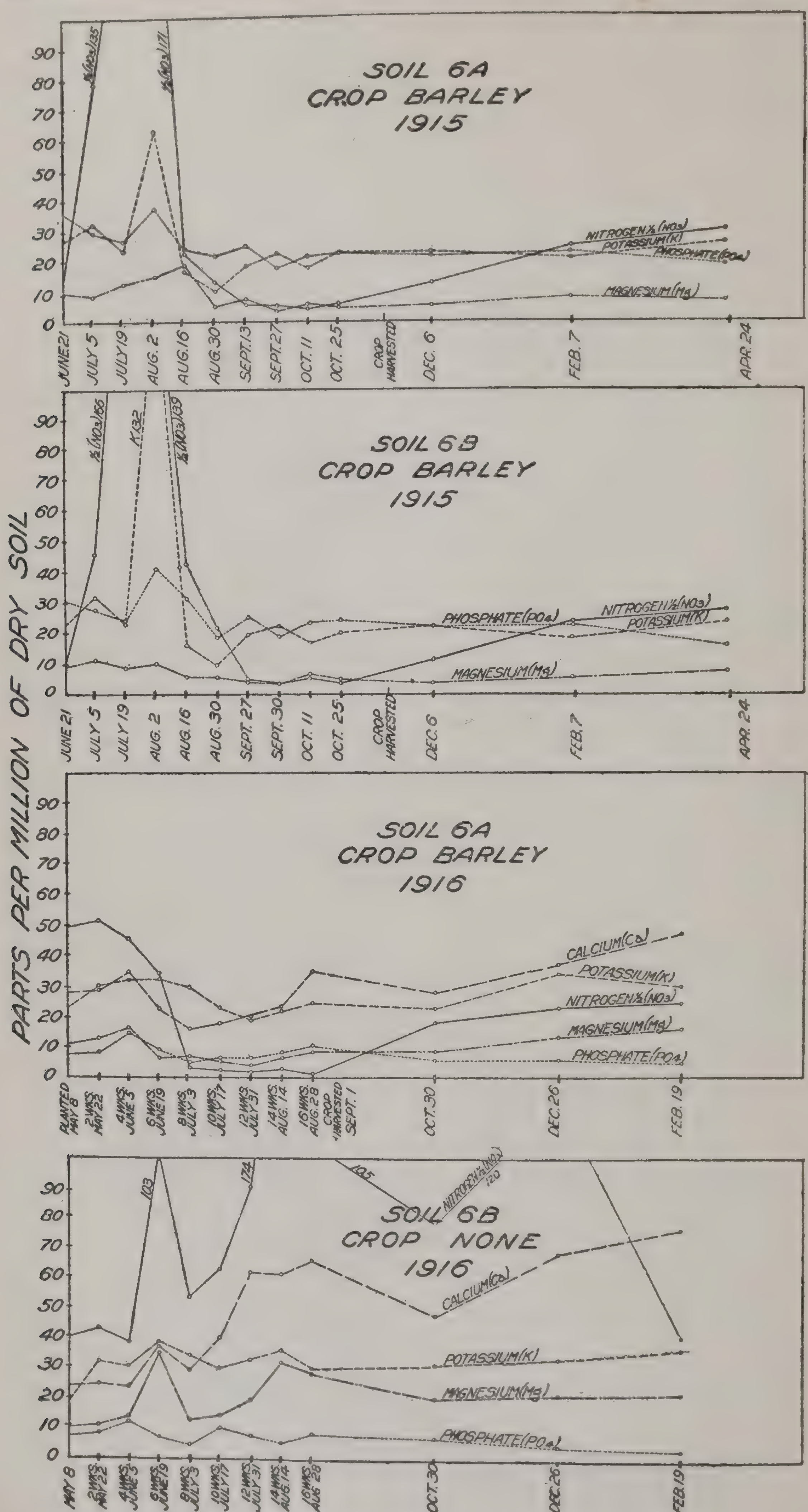

FIG. 13.—Graphs of the seasonal studies of the water extract of soil 6, Yolo clay loam .

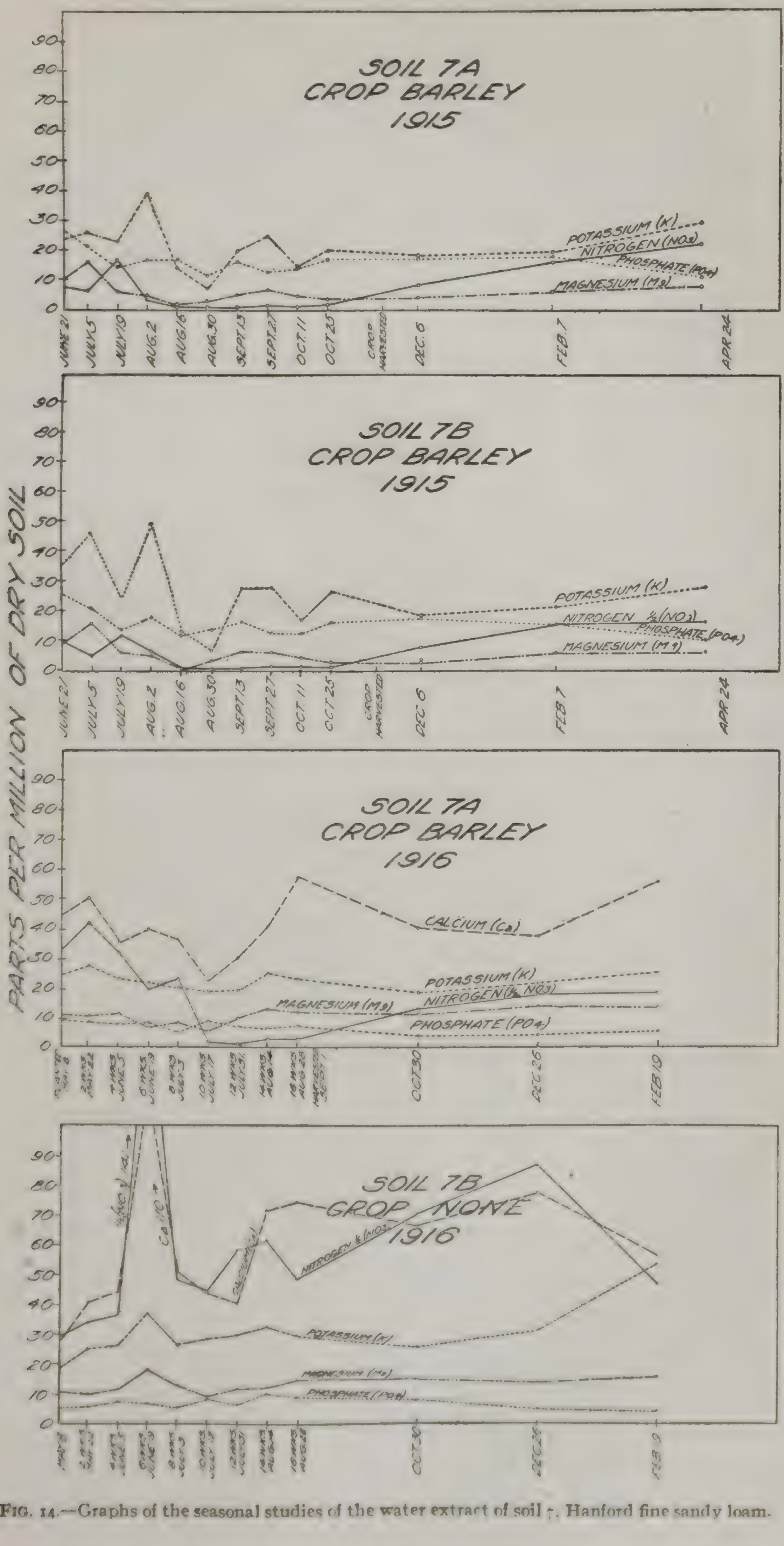

FIG. 14.—Graphs of the seasonal studies of the water extract of soil 7, Hanford fine sandy loam.

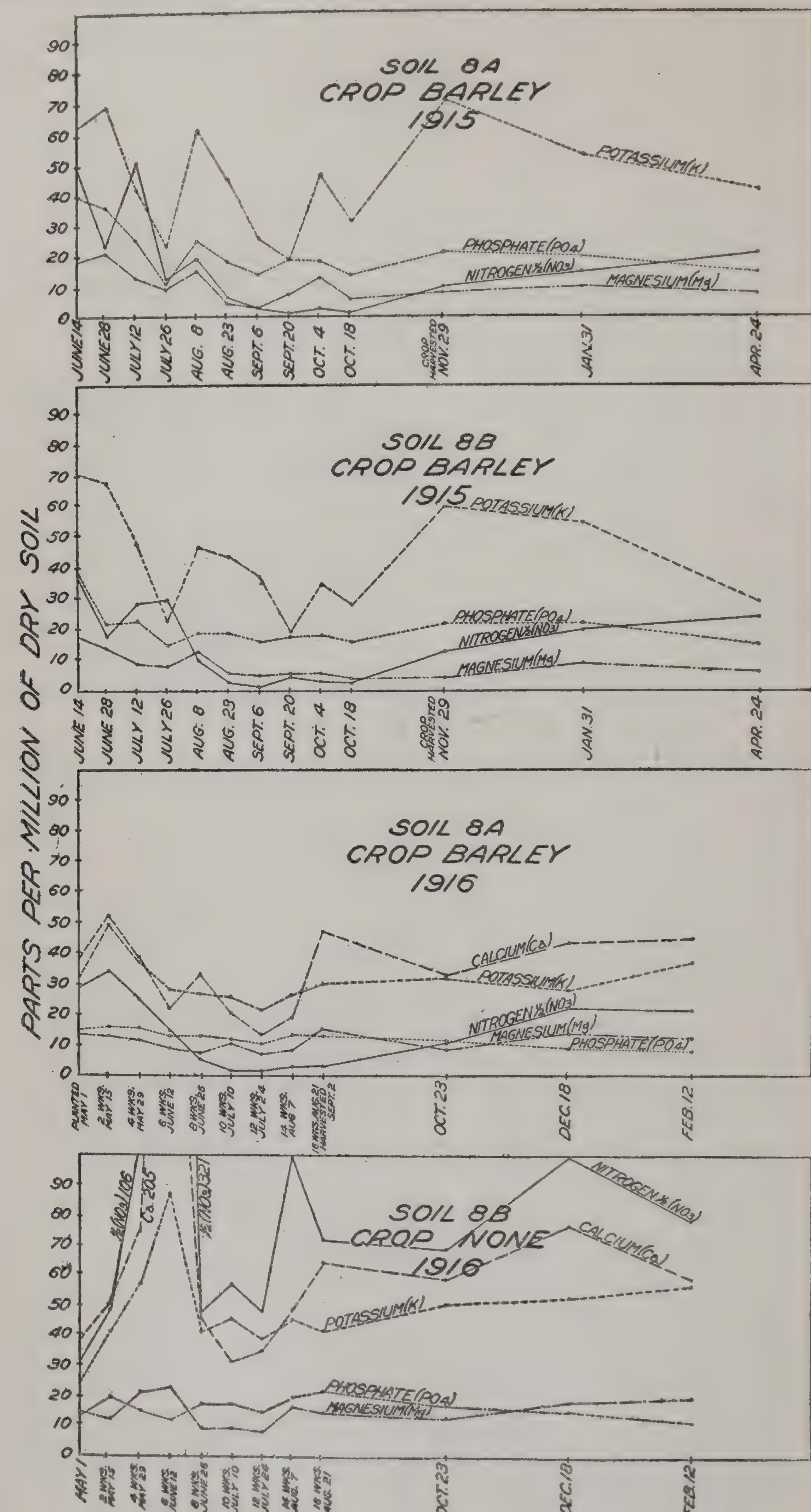

FIG. 15.—Graphs of the seasonal studies of the water extract of soil 8, Fresno fine sandy loam.

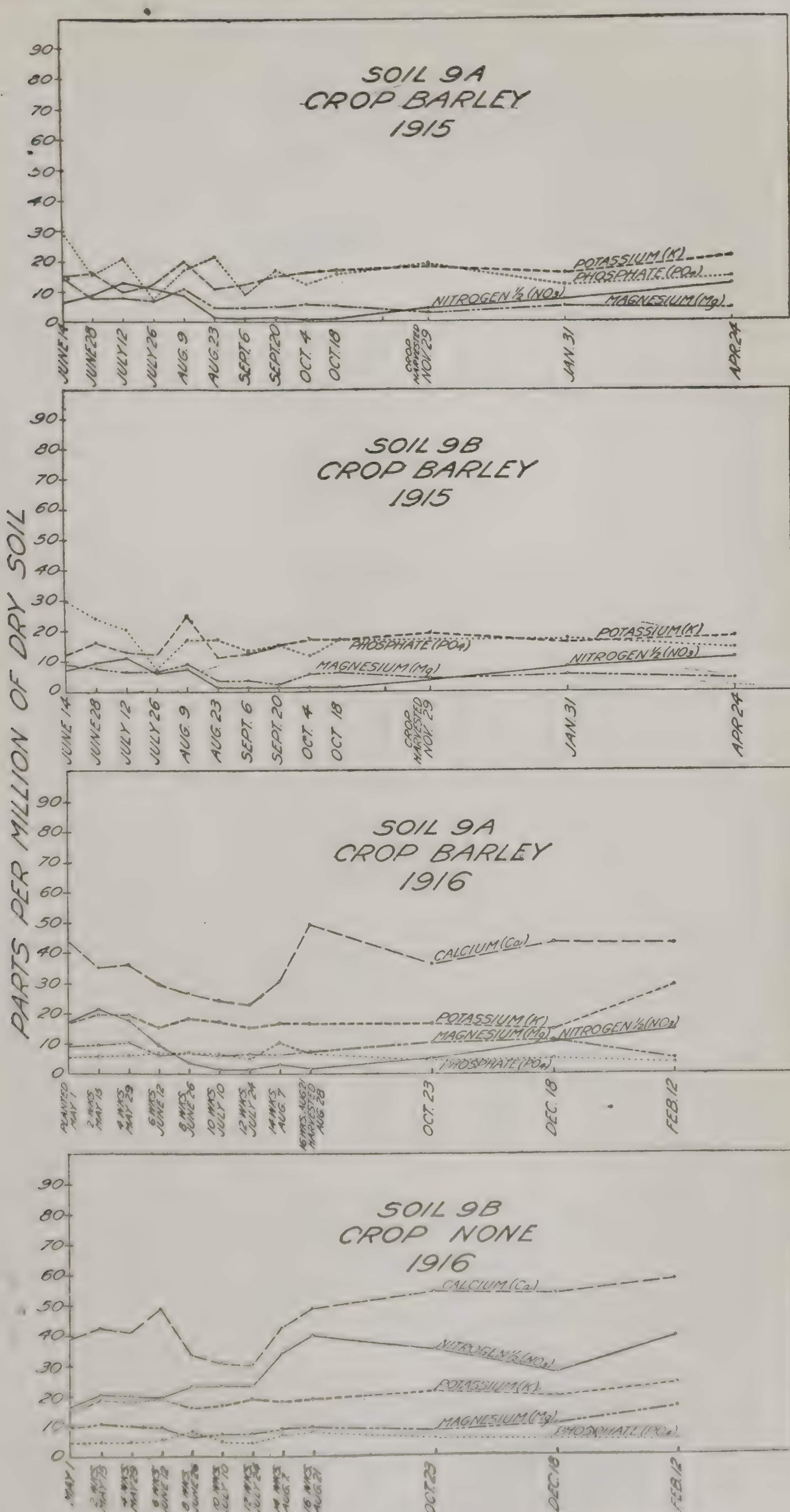

Fig. 16.—Graphs of the seasonal studies of the water extract of soil 9, Kimball fine sandy loam.

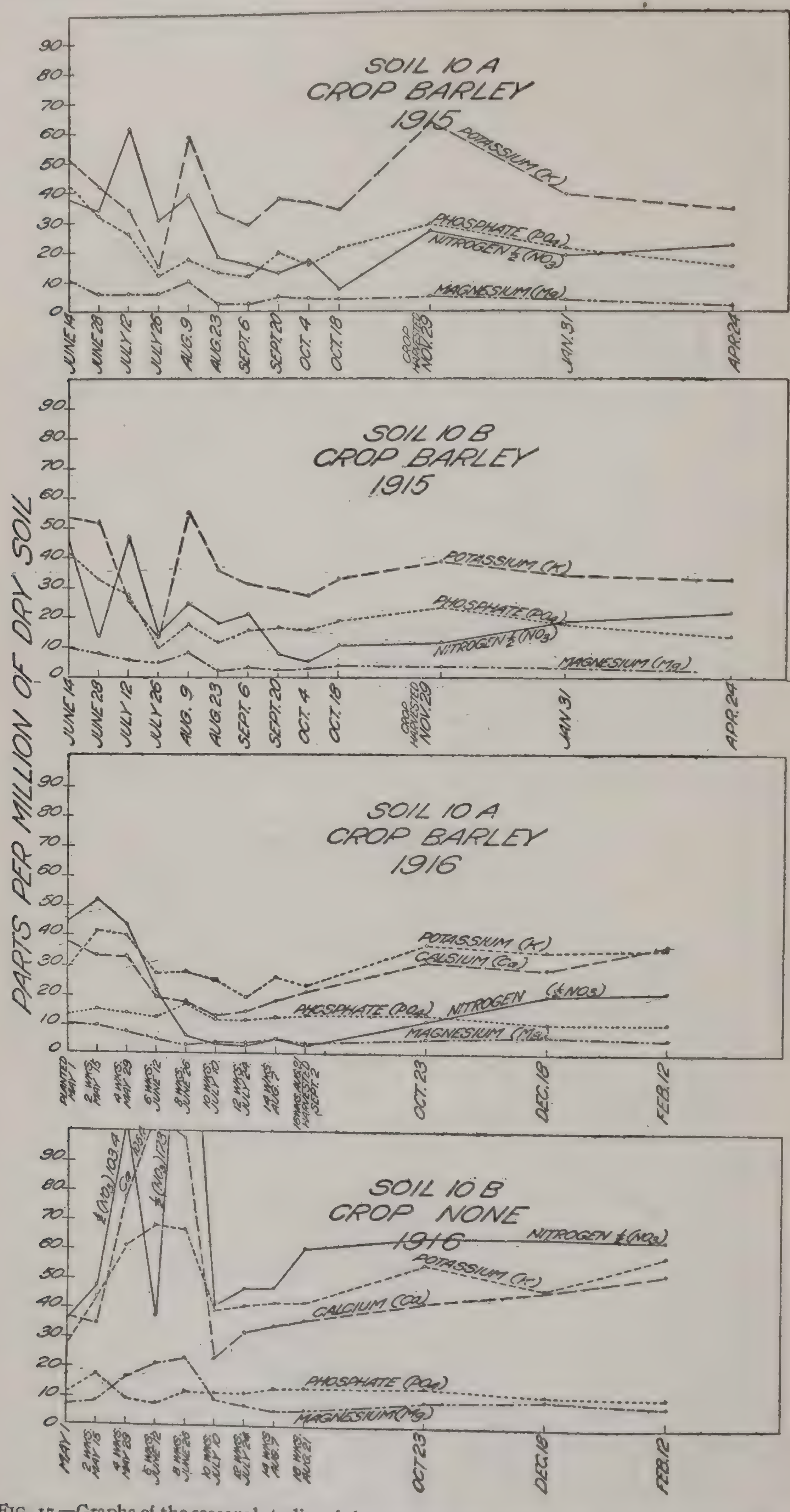

FIG. 17.—Graphs of the seasonal studies of the water extract of soil 10, Tejunga fine sandy loam.

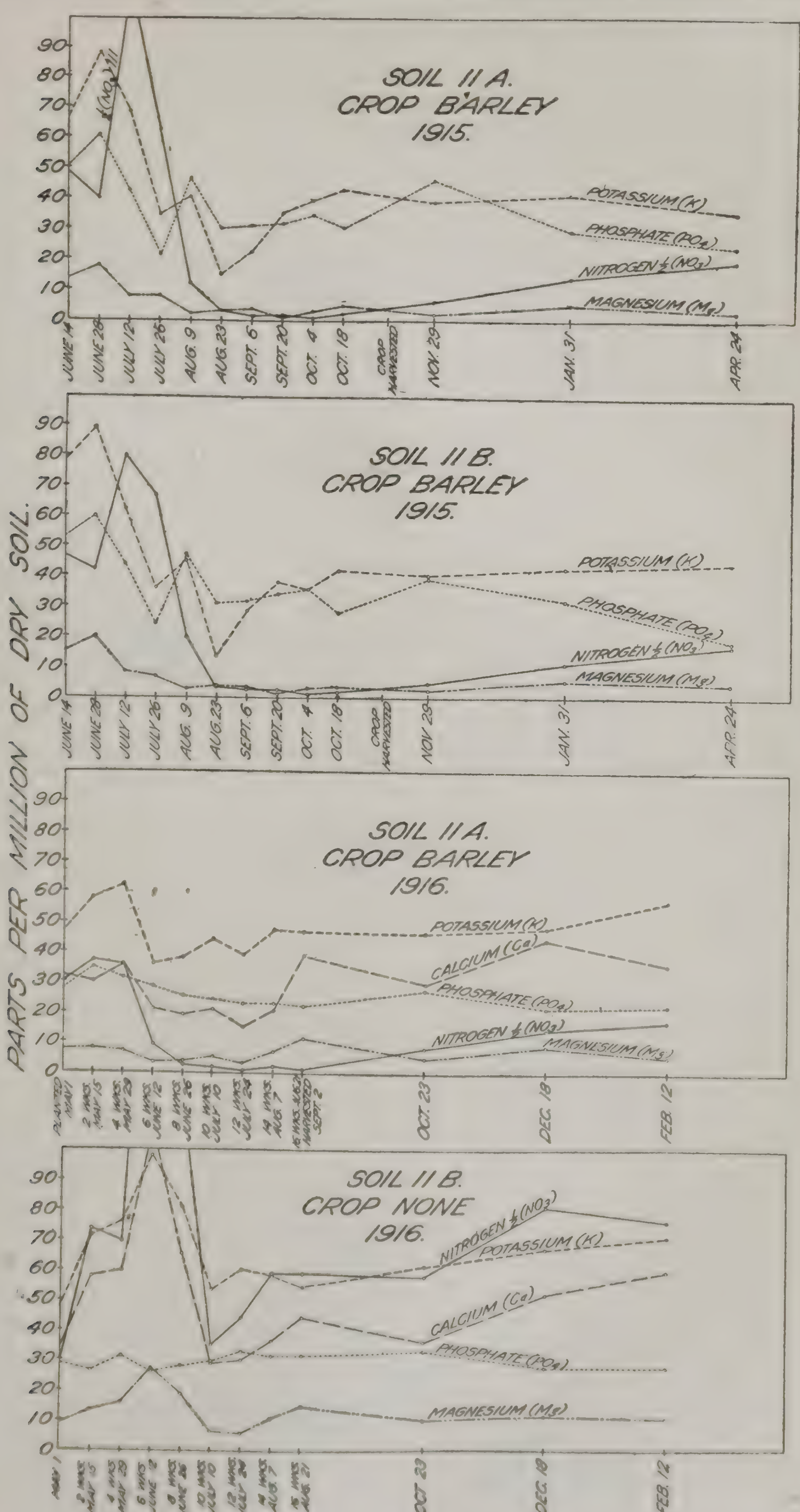

FIG. 18.—Graphs of the seasonal studies of the water extract of soil 11, Madera fine sandy loam.

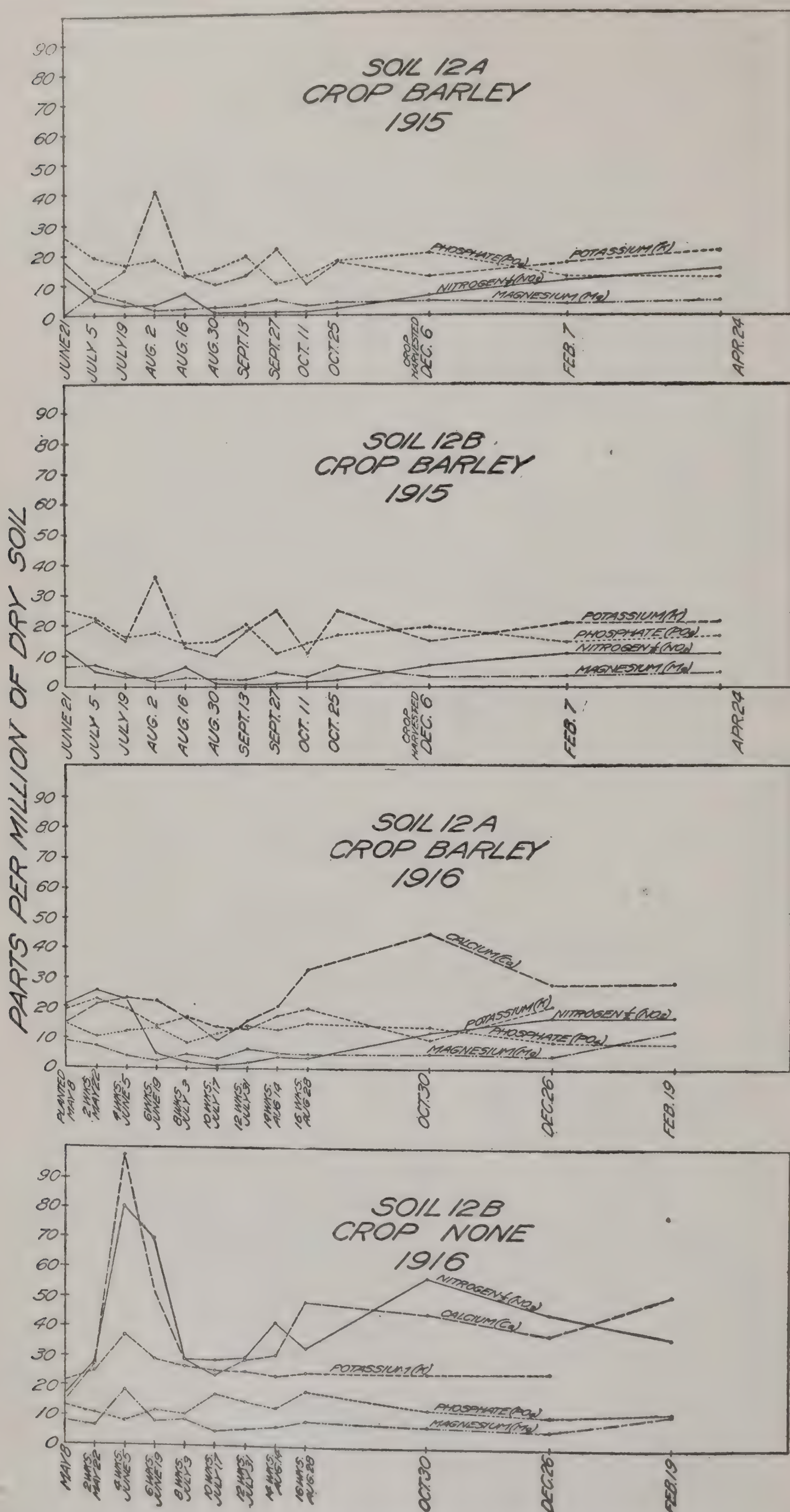

FIG. 19.—Graphs of the seasonal studies of water extract of soil 12, Arnold fine sandy loam.

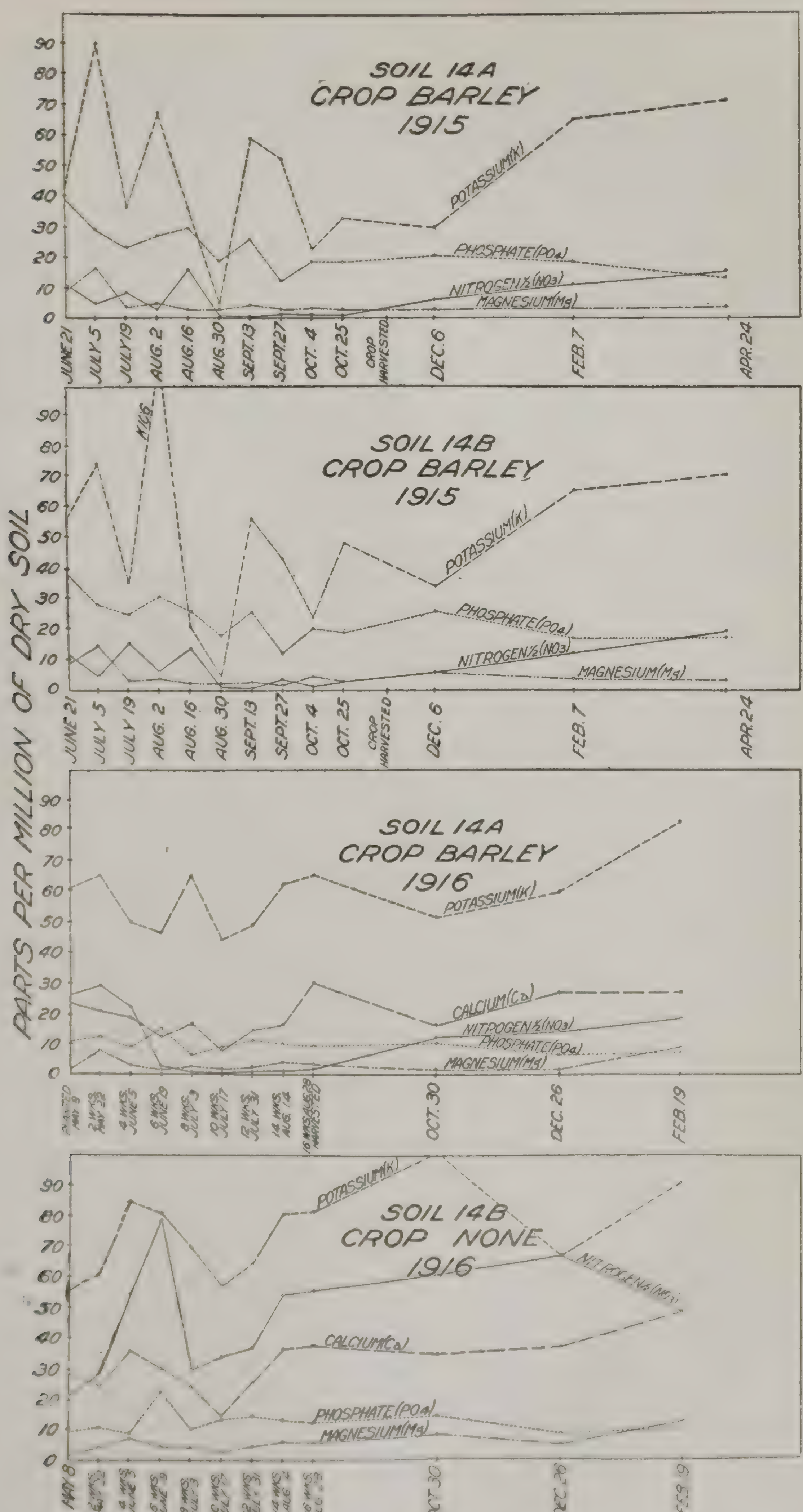

FIG. 20.—Graphs of the seasonal studies of the water extract of soil 14, Standish fine sandy loam.

SECOND SEASON, 1916

In the season of 1916 one container of each soil was again planted to the same strain of Beldi barley, and the duplicate was at all times treated in the same manner, except that no crop was grown upon it. One week before planting, the top soil to a depth of 8 or 10 inches was forked up and put in an excellent state of tilth. The soils were again sampled every two weeks from the time of planting in May until the crop was harvested in August. During the succeeding fall and winter samples were taken at approximately 8-week intervals.

The analyses of the water extracts were performed by the modified methods which have been previously outlined. During the latter portion of the sampling season cooperative work was performed by Hoagland on the samples, using the freezing-point method.

An extremely uniform stand of barley was obtained in all containers. Shortly after sprouting, the plants were thinned to one vigorous seedling. The growth throughout the season was steady and vigorous. A diagrammatic representation of the height at each soil-sampling period is given in figure 21. It will be seen that the most rapid period of growth was from the fourth to the tenth week. By that time the plants had almost attained their full height, and the heads of grain were beginning to be formed. At the close of the twelfth week the maximum growth in height had been attained. Five weeks' additional time was required for complete development and ripening. It was interesting to note that in these studies, where moisture was never a limiting factor, the growth in height was extremely uniform, even though the dry matter produced did show great differences.

The results of the crop yield in total crop and grain are given in Table IX. The grain yield has again been calculated to pounds and bushels per acre, and the percentage of variation from the maximum yield is shown.

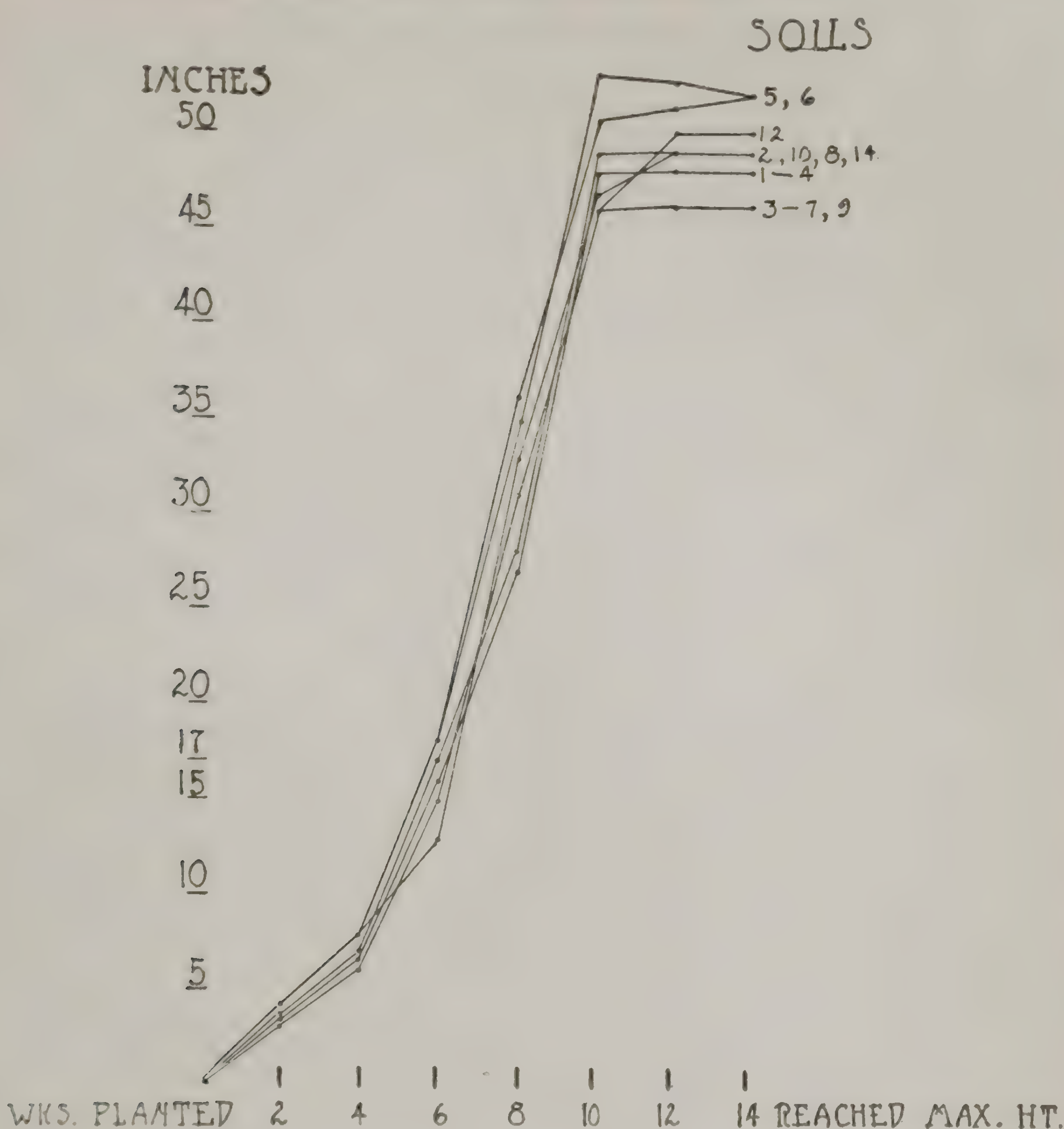

FIG. 21.—Graphs of the growth of crops in height, season of 1916.

TABLE IX.—*Crop yield in 1916*

Soil.	Total yield of air-dry grain and straw.	Grain.	Grain (pounds per acre).	Grain (bushels per acre).	Variation from maximum yield of grain.
Yolo silty clay loam:	*Grams.*	*Grams.*			*Per cent.*
No. 1A	1,474	675	5,192	86.5	2.2
No. 2A	1,530	655	5,038	84.0	5.0
No. 3A	970	439	3,377	56.2	36.4
No. 4A	1,252	549	4,223	70.4	20.4
No. 5A	1,652	690	5,307	88.4	.0
Yolo clay loam, 6A	1,523	670	5,153	85.9	2.8
Hanford fine sandy loam, 7A	1,414	541	4,161	69.4	22.5
Fresno fine sandy loam, 8A	1,719	679	5,222	87.0	1.6
Kimball fine sandy loam, 9A	946	357	2,746	45.8	48.2
Tejunga fine sandy loam, 10A	1,266	551	4,238	70.6	20.1
Madera fine sandy loam, 11A	1,547	625	4,807	80.1	9.4
Arnold fine sandy loam, 12A	970	390	3,000	50.0	43.4
Standish fine sandy loam, 14A	1,464	630	4,846	80.8	8.6

The figures obtained in the preceding season for the mean and maximum variation between duplicates will be used in order to find what will constitute a significant difference in yield. By taking the mean variation as the standard, soils 5A, 8A, 1A, 6A, and 2A are to be considered equally productive, while 11A and 14A are slightly lower. If, however, the conservative figure of the maximum variation between duplicates is used, it will be seen that No. 11A and 14A will also fall in the group of the highest yield. These relations are presented diagrammatically in figure 7.

It will be seen that six soils were significantly lower than the group of highest productivity. Of those No. 10A, 4A, and 7A were practically equal, while 3A, 12A, and 9A were distinctly low in yield.

The results of the water extractions are again presented in the form of graphs. In figures 8 to 20 the plot marked "A" is in each case the planted portion, while the uncropped duplicate is called "B." Some extremely striking differences are exhibited by these graphs.

In the planted soils the water-soluble nutrients at the beginning of the season, either remained on practically the same level or increased slightly for the first four weeks. Then, without exception, the nitrogen commenced to decrease rapidly and was followed in a smaller degree by the calcium and potash, and very slightly by the magnesium. The contrast shown by the unplanted soil was equally uniform in nature, though variable in the extent of the effect. In almost all the soils the effect of the cultivation was to cause a considerable liberation of soluble nutrients and, though there was later shown to be a depression from this high figure, yet the general range of all the nutrients except the phosphates continued to be higher in the uncropped soil.

The soils which did not show this stimulation and liberation of nutrients were No. 3B, 9B, and 12B. These were the least productive soils of the group and also had the lowest range of soluble nutrients.

It is extremely significant that this same period of high soluble nutrients in the uncropped soils corresponds to the period of lowered nutrients in the cropped duplicates. It was also the period, as may be observed in figure 21, in which the plants were making their most rapid growth.

The one compound which did not exhibit this liberation of excess nutrients in the uncropped soil was the phosphate. There were large differences between the amounts present in various soils, but for any one soil the amount was practically constant in the cropped and uncropped plot. It was the only soil nutrient studied which behaved in this manner.

All the above differences between the planted and unplanted soils which have been noted are greater than can be accounted for by the probable factor of error which has been determined in an earlier portion of the study. The maximum figure for any nutrient in the concentration found is 10 per cent, and that applies only to potassium and calcium. On applying this correction plus or minus to the periods of greatest de-

pression, it is clear that this will not account for the differences noted. With phosphates the probable maximum is 4 to 6 per cent, while with magnesium it would vary from 6 to 10 per cent. All these corrections fail to change significantly the figures recorded.

At two periods during the season of 1916 daily studies of the water extract were made with soil 8, and at one period with soil 1. The result of these tend to show that considerable fluctuations may take place from day to day. The results of these two studies are plotted in figures 22 to 24. It will be seen that the fluctuations in general occur simultaneously in both containers and so will not alter the relationship of the graphs for the planted and unplanted soils. They show, however, that small differences in the range of the graphs can not be considered sig-

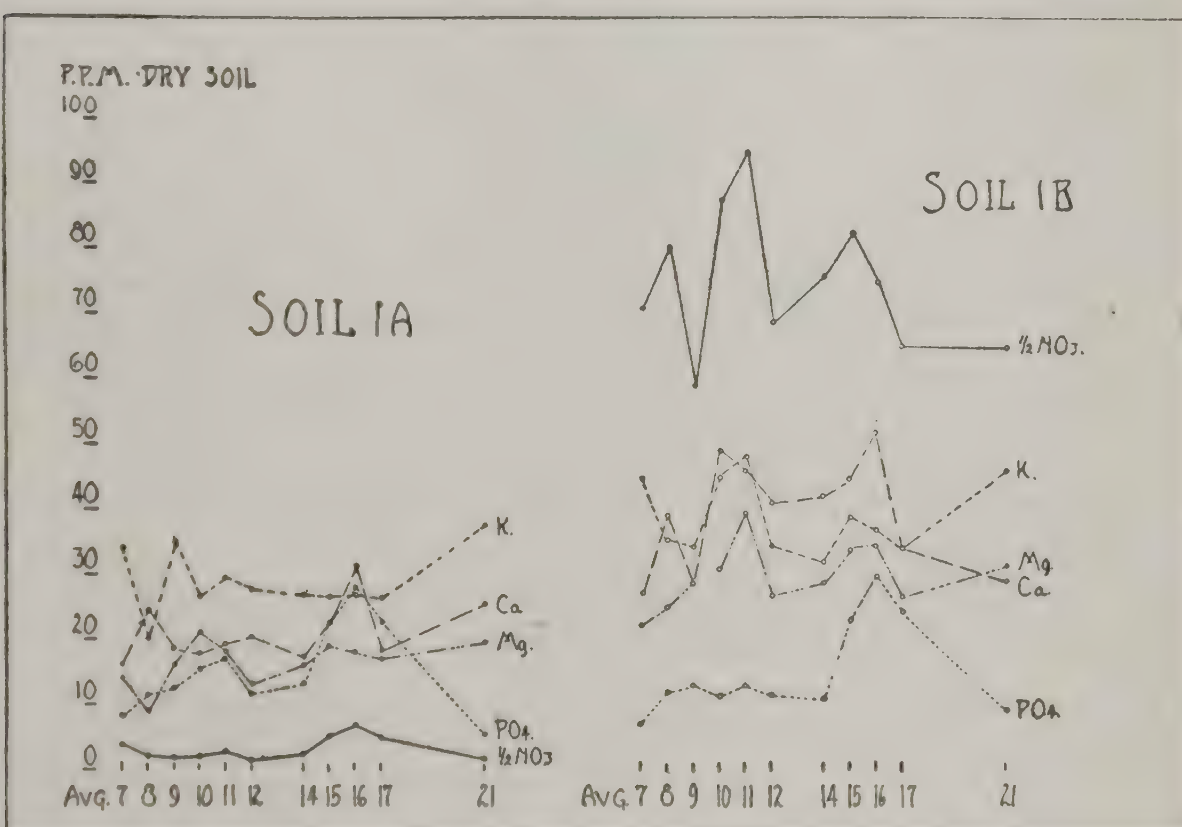

FIG. 22.—Graphs of the daily studies of the water extract of soils 1A and 1B, season of 1916.

nificant. The general range of the graphs is believed to represent an average figure, but small variations between soils should always be disregarded. This would also indicate that an expression of the differences in water-soluble material can only be given by a series of related observations.

DISCUSSION AND DEDUCTIONS

In the foregoing studies it is believed that the limitations as well as the possibilities of the experimental methods have been pointed out. It is earnestly desired that no deductions should be drawn which are not conservatively justified by the data presented.

It should be noted that the results have been obtained on 13 soils which comprise only two distinct soil types. These types are, however,

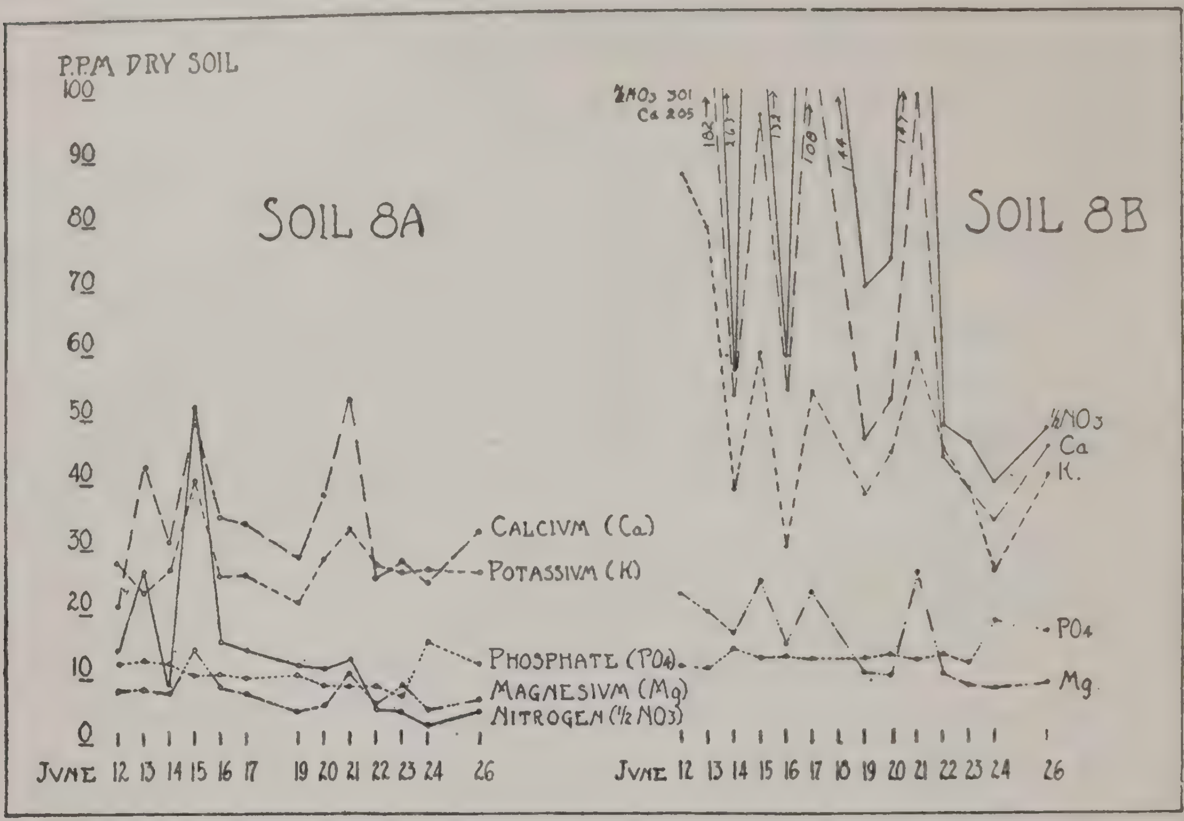

FIG. 23.—Graphs of the daily studies of the water extract of soils 8A and 8B, July, 1916.

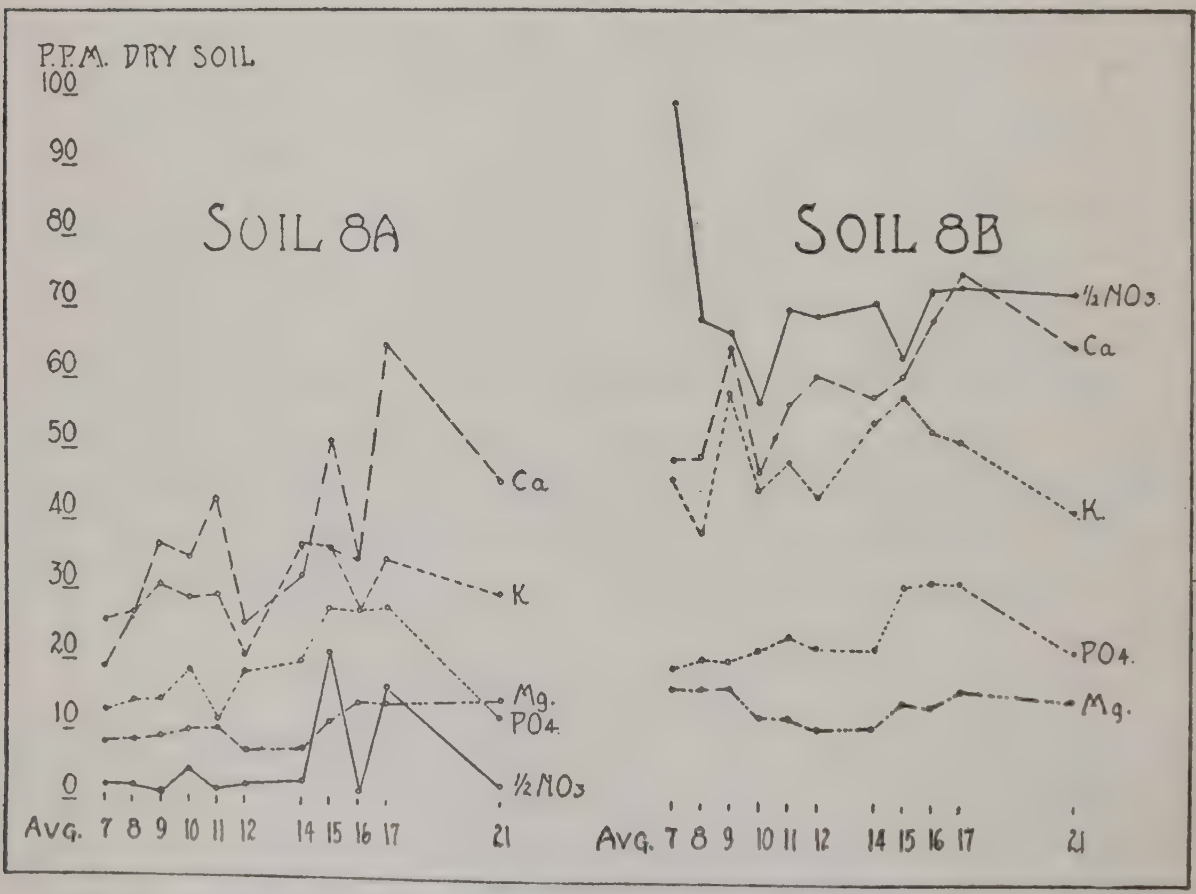

FIG. 24.—Graphs of the daily studies of the water extract of soils 8A and 8B, August, 1916.

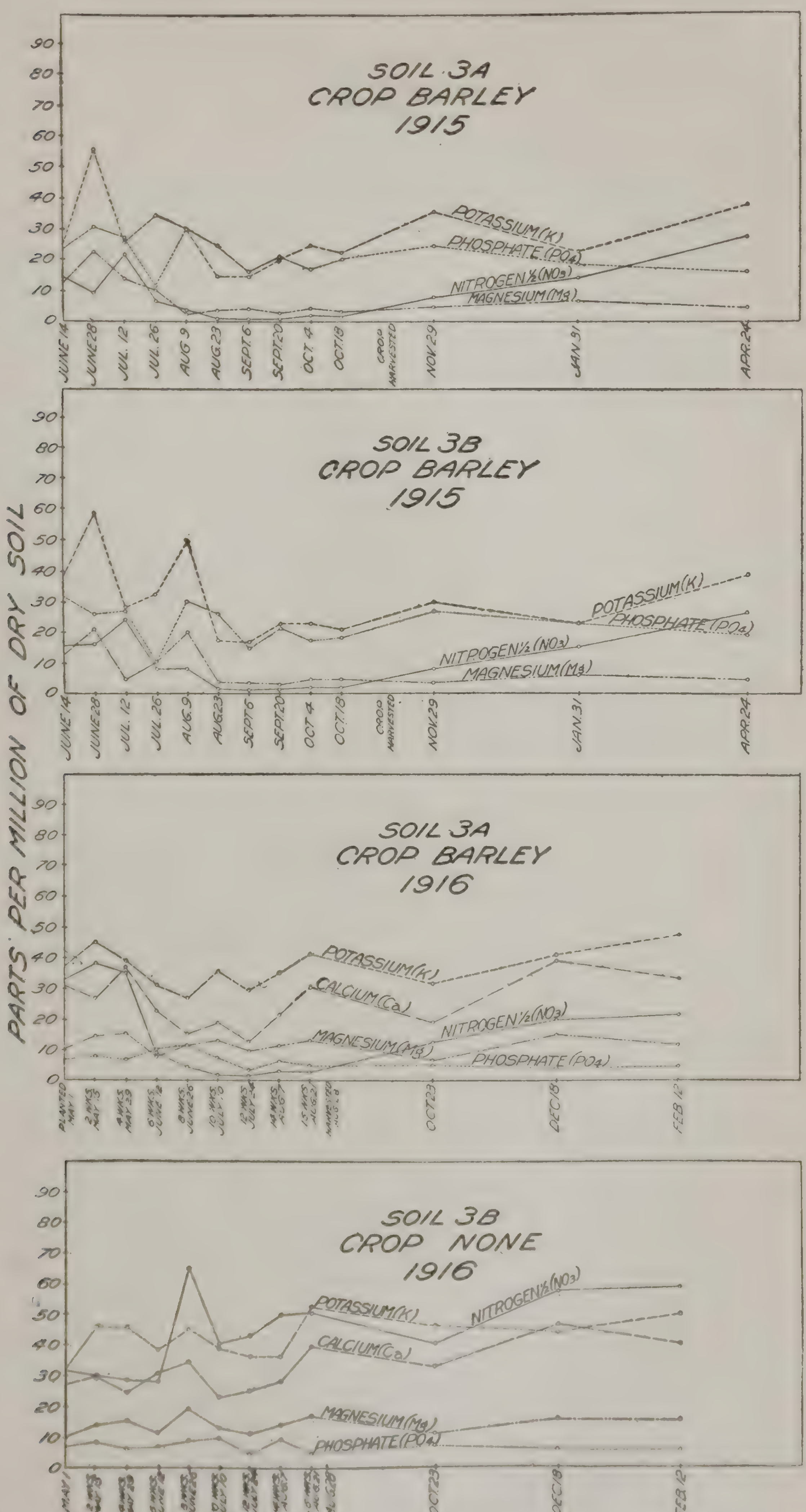

FIG. 10.—Graphs of the seasonal studies of the water extract of soil 3. Yolo silty clay loam.

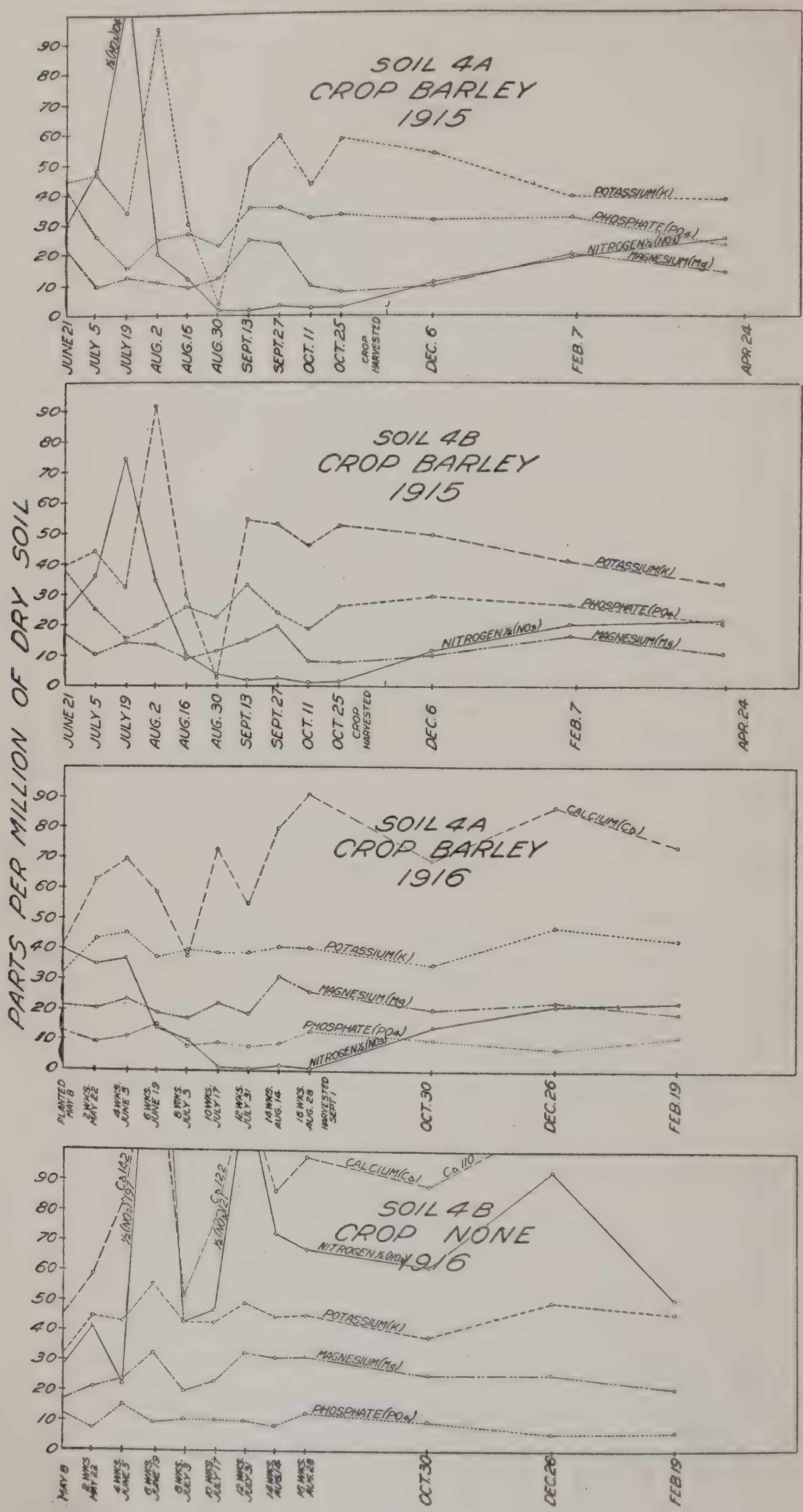

FIG. 11.—Graphs of the seasonal studies of the water extract o fsoil 4, Yo'o silty clay loam.

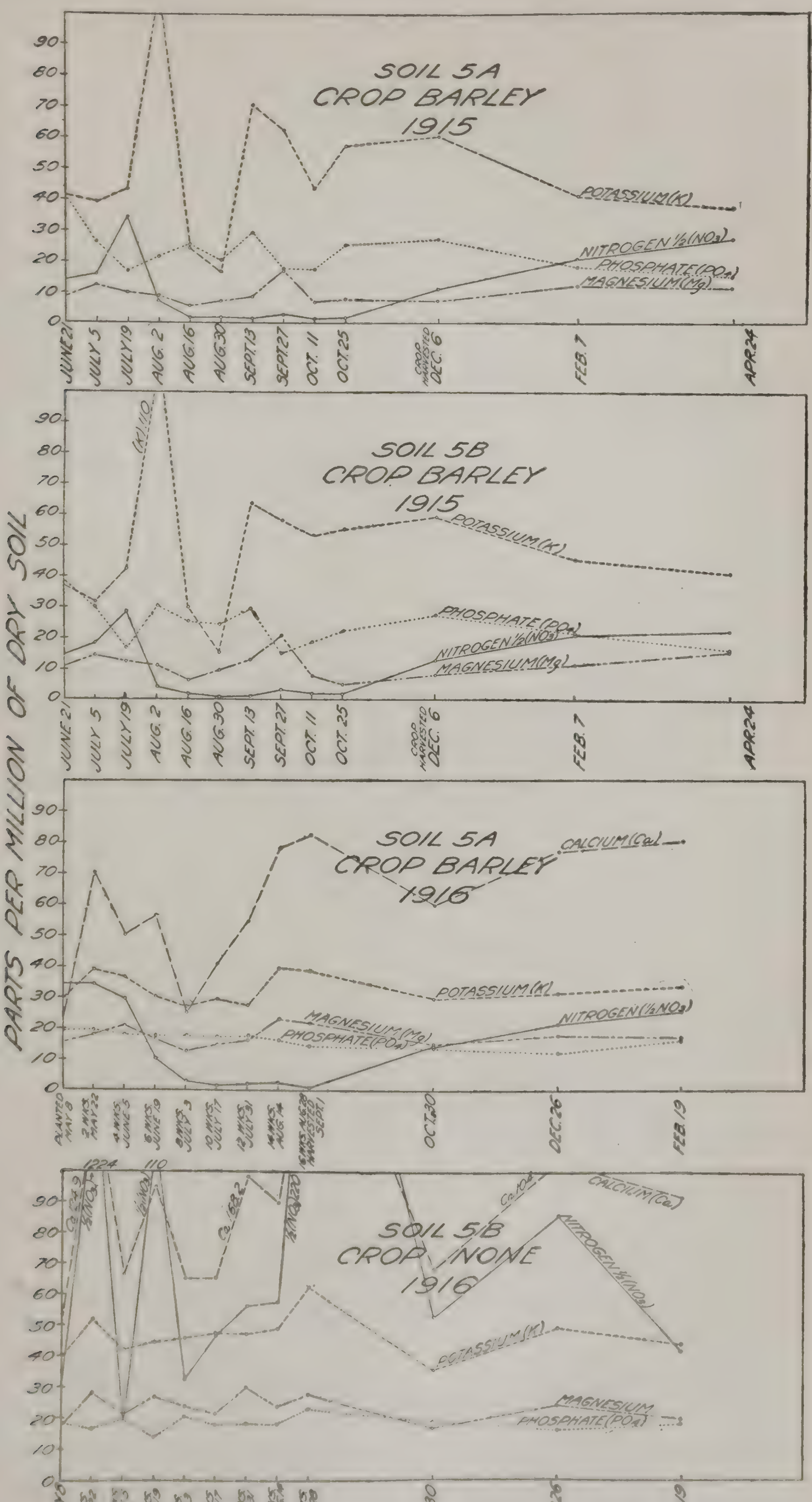

FIG. 12.—Graphs of the seasonal studies of the water extract of soil 5, Yolo silty clay loam.

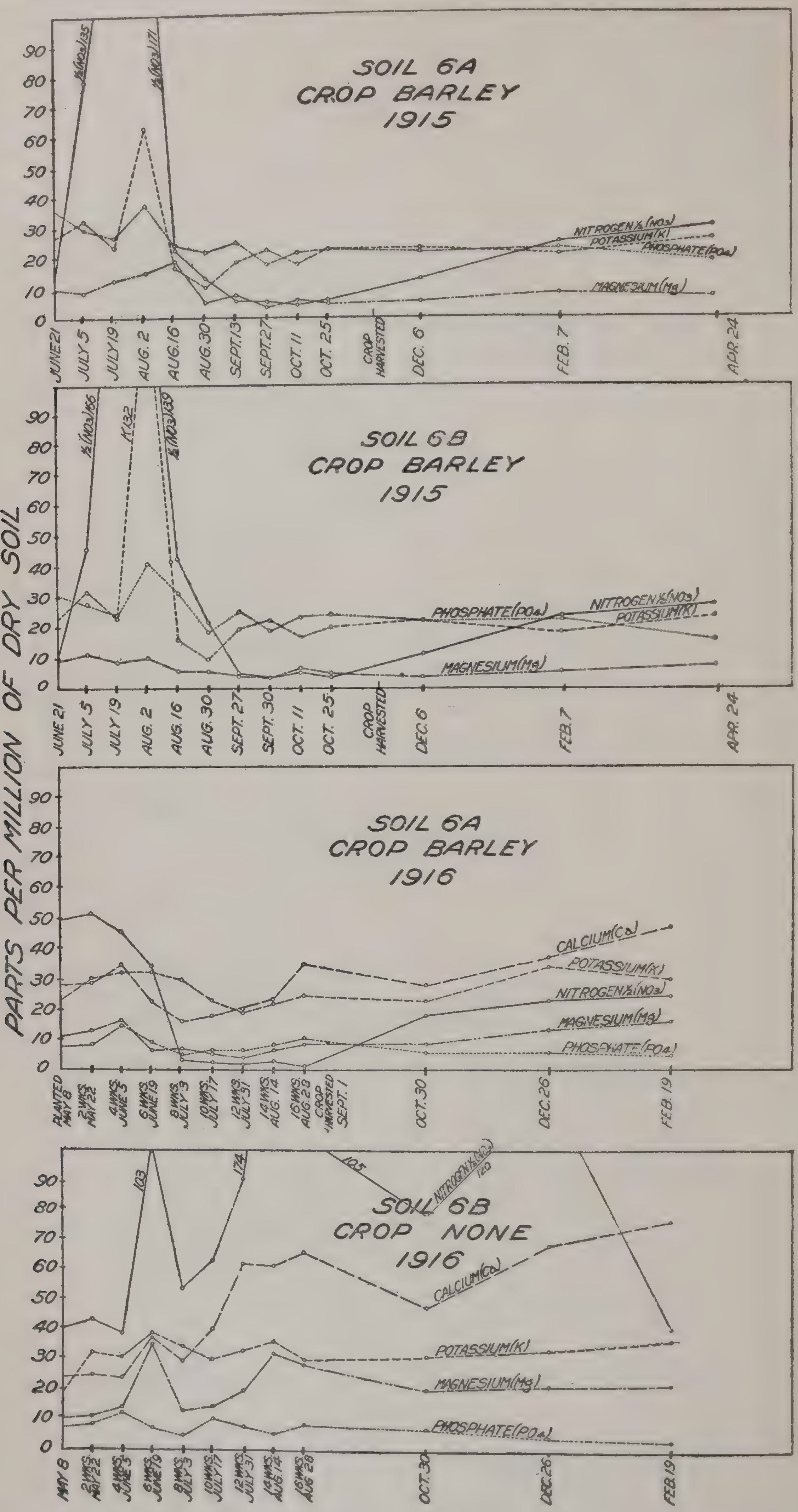

FIG. 13.—Graphs of the seasonal studies of the water extract of soil 6, Yolo clay loam.

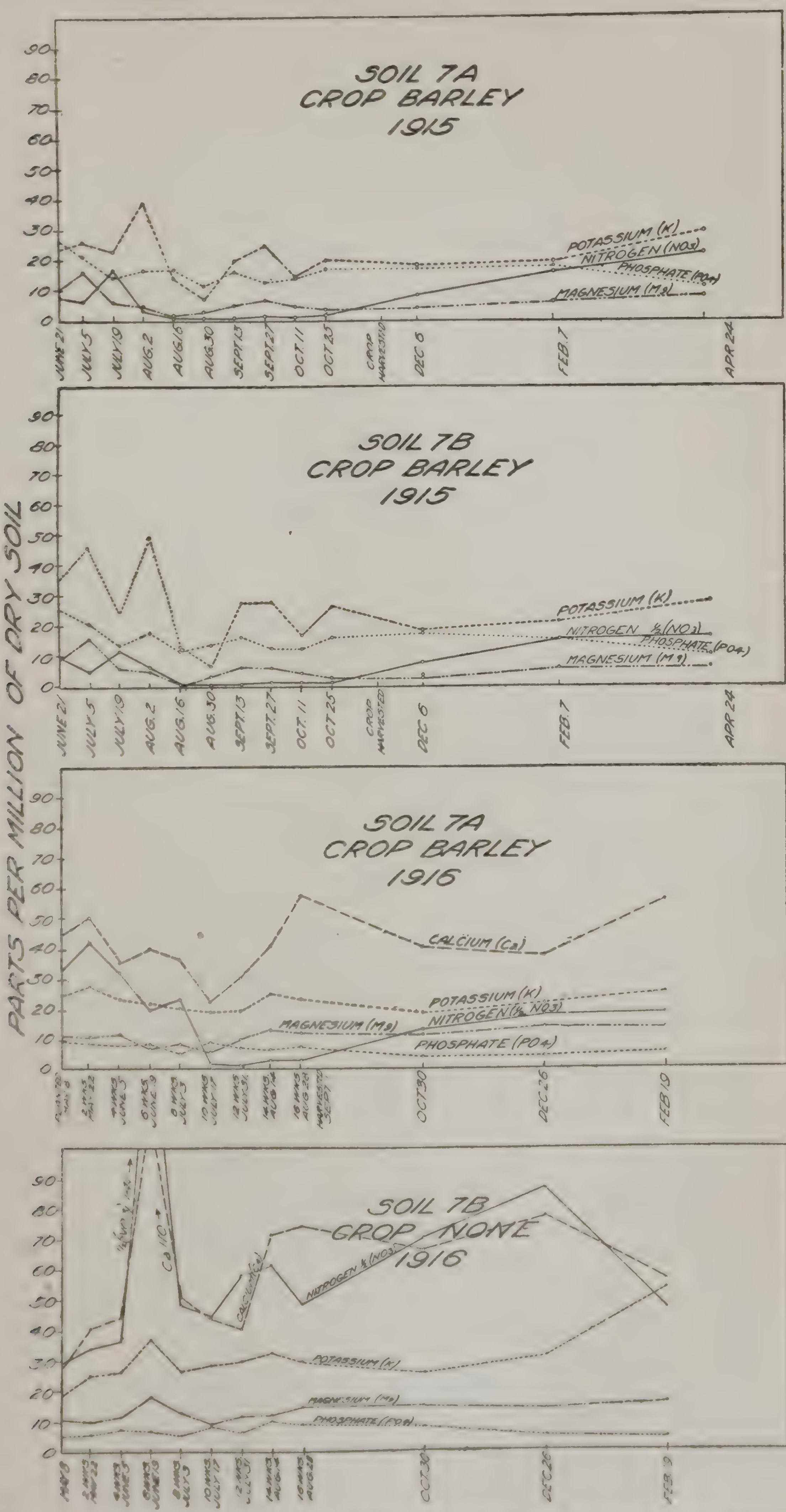

FIG. 14.—Graphs of the seasonal studies of the water extract of soil 7, Hanford fine sandy loam.

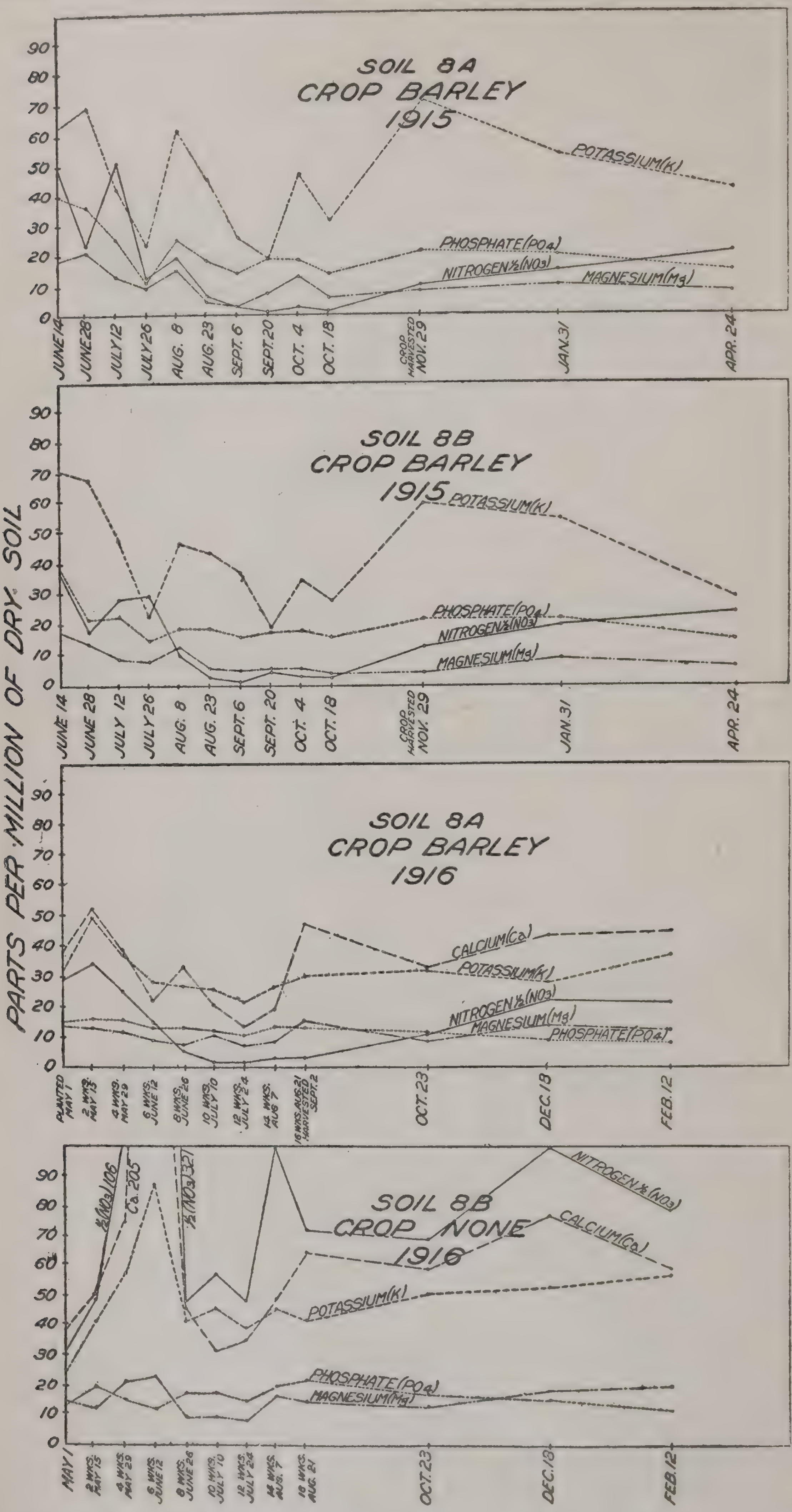

FIG. 15.—Graphs of the seasonal studies of the water extract of soil 8, Fresno fine sandy loam.

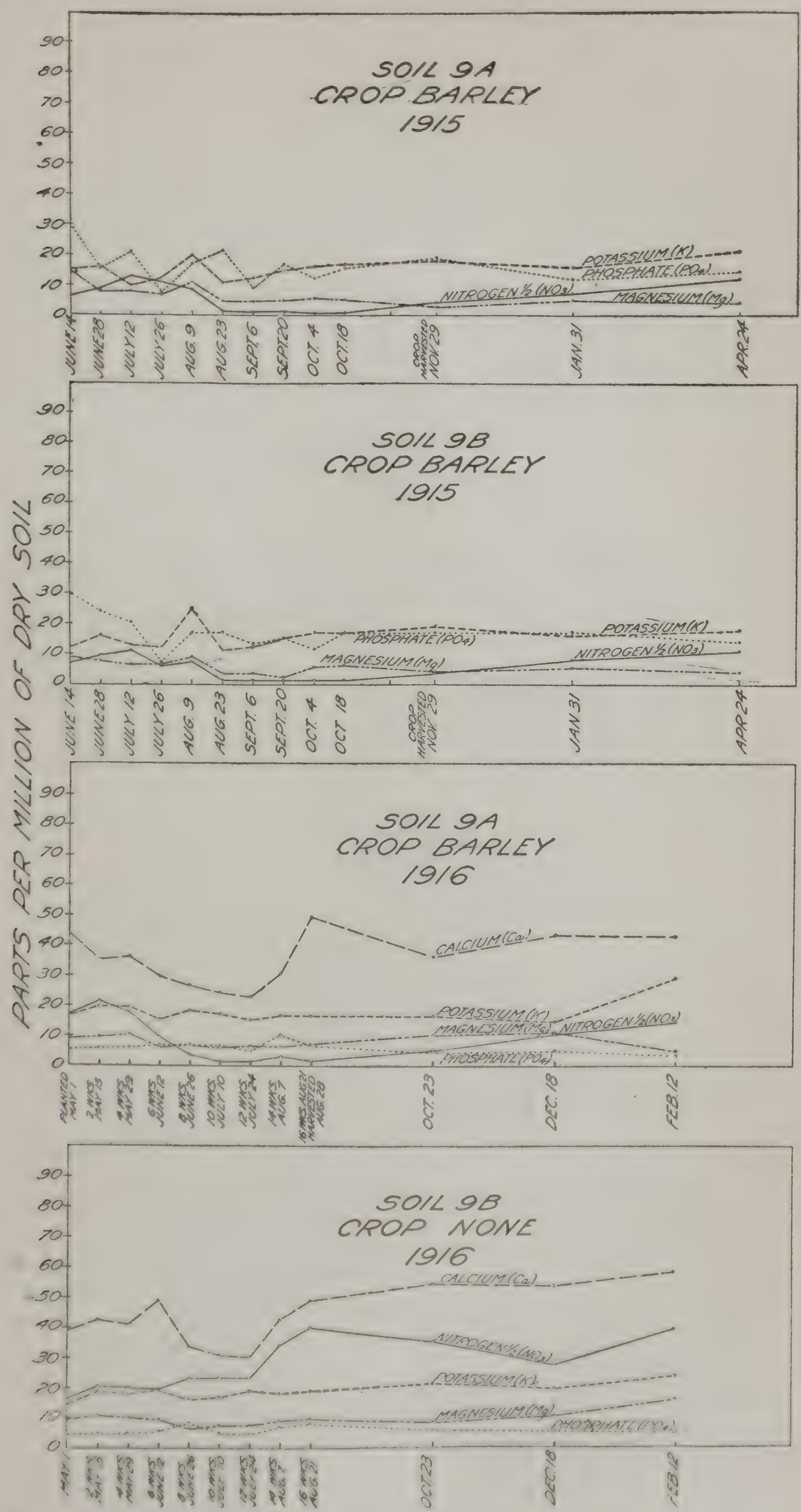

FIG. 16.—Graphs of the seasonal studies of the water extract of soil 9, Kimball fine sandy loam.

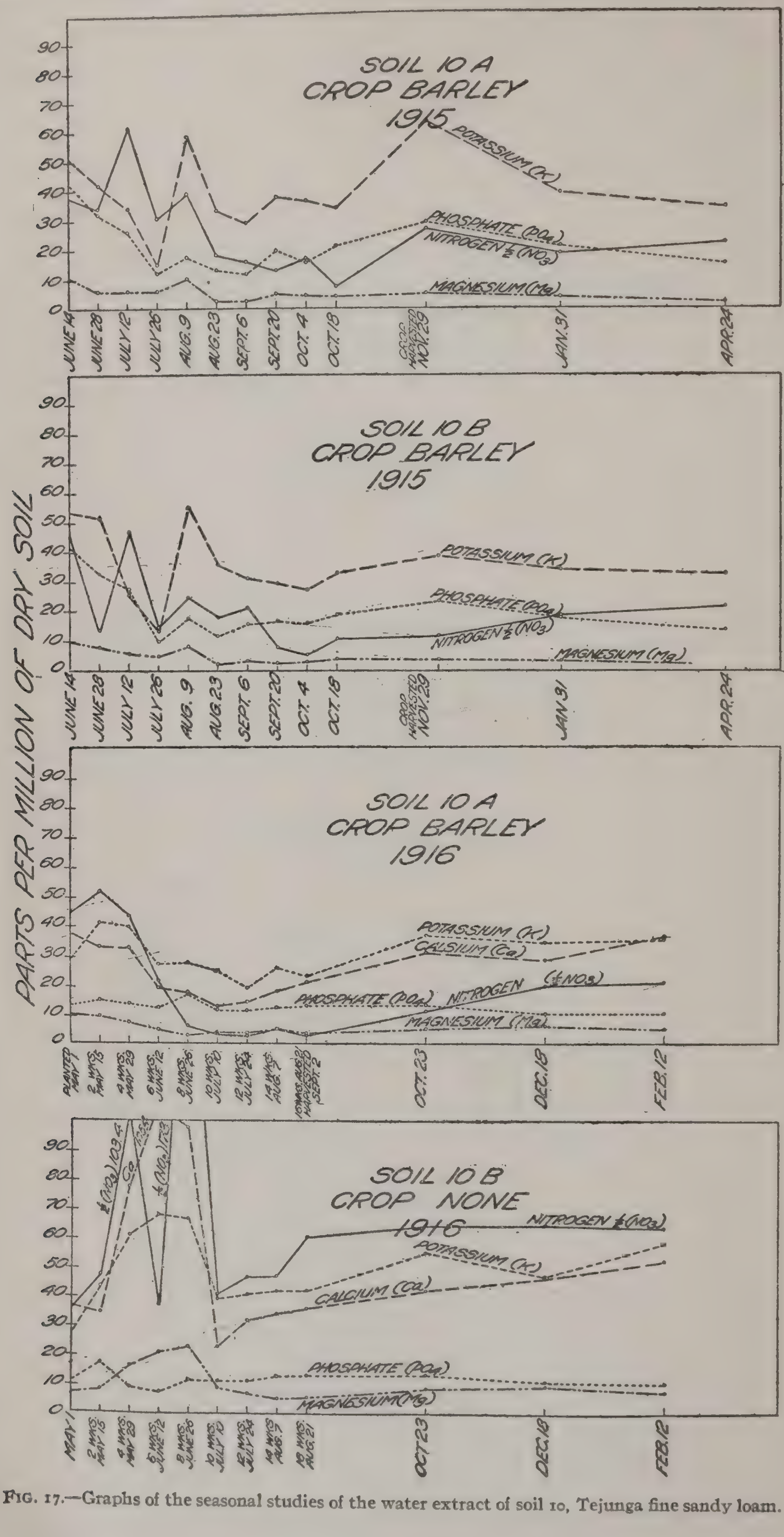

FIG. 17.—Graphs of the seasonal studies of the water extract of soil 10, Tejunga fine sandy loam.

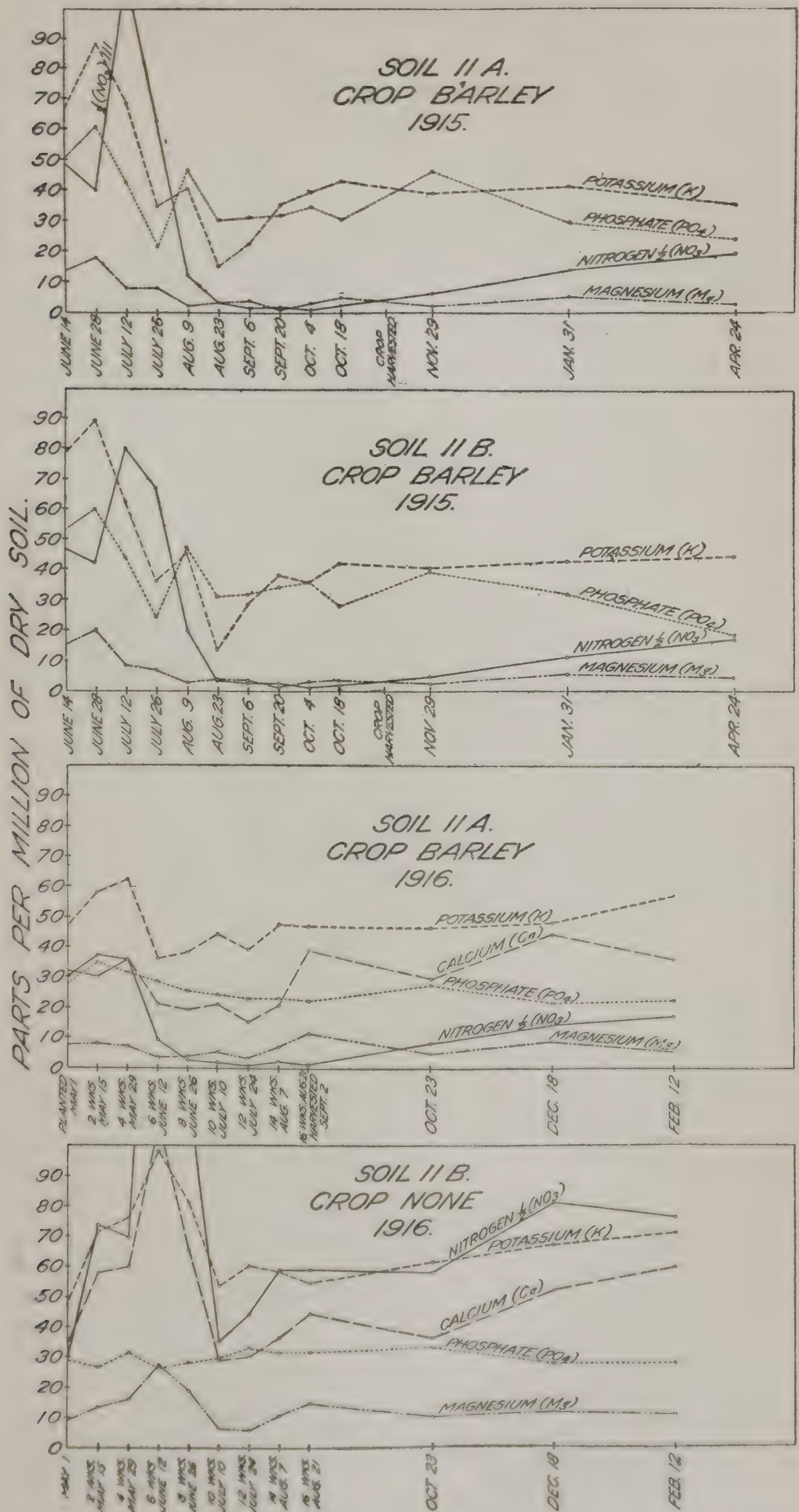

FIG. 18.—Graphs of the seasonal studies of the water extract of soil 11, Madera fine sandy loam.

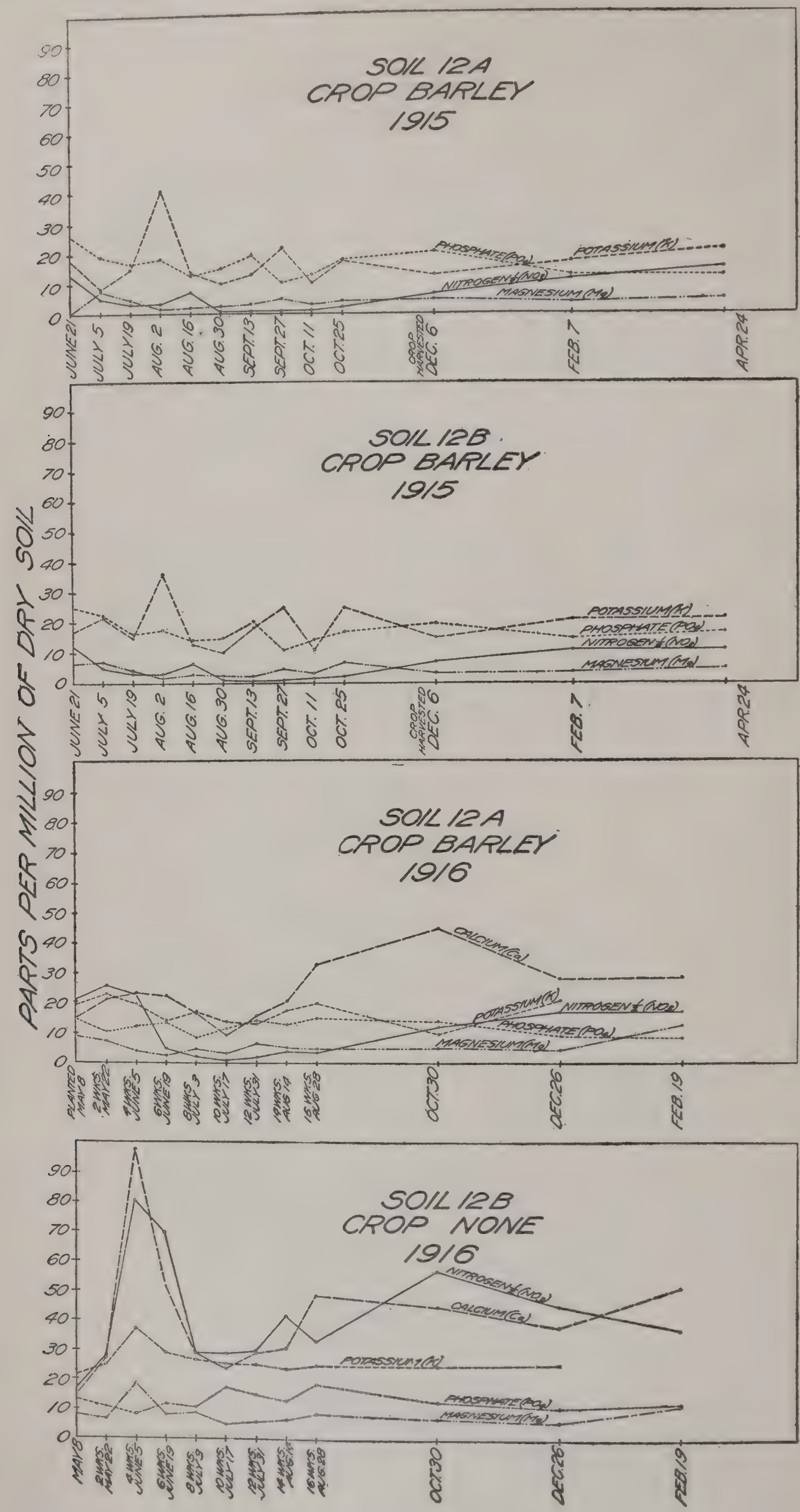

FIG. 19.—Graphs of the seasonal studies of water extract of soil 12, Arnold fine sandy loam.

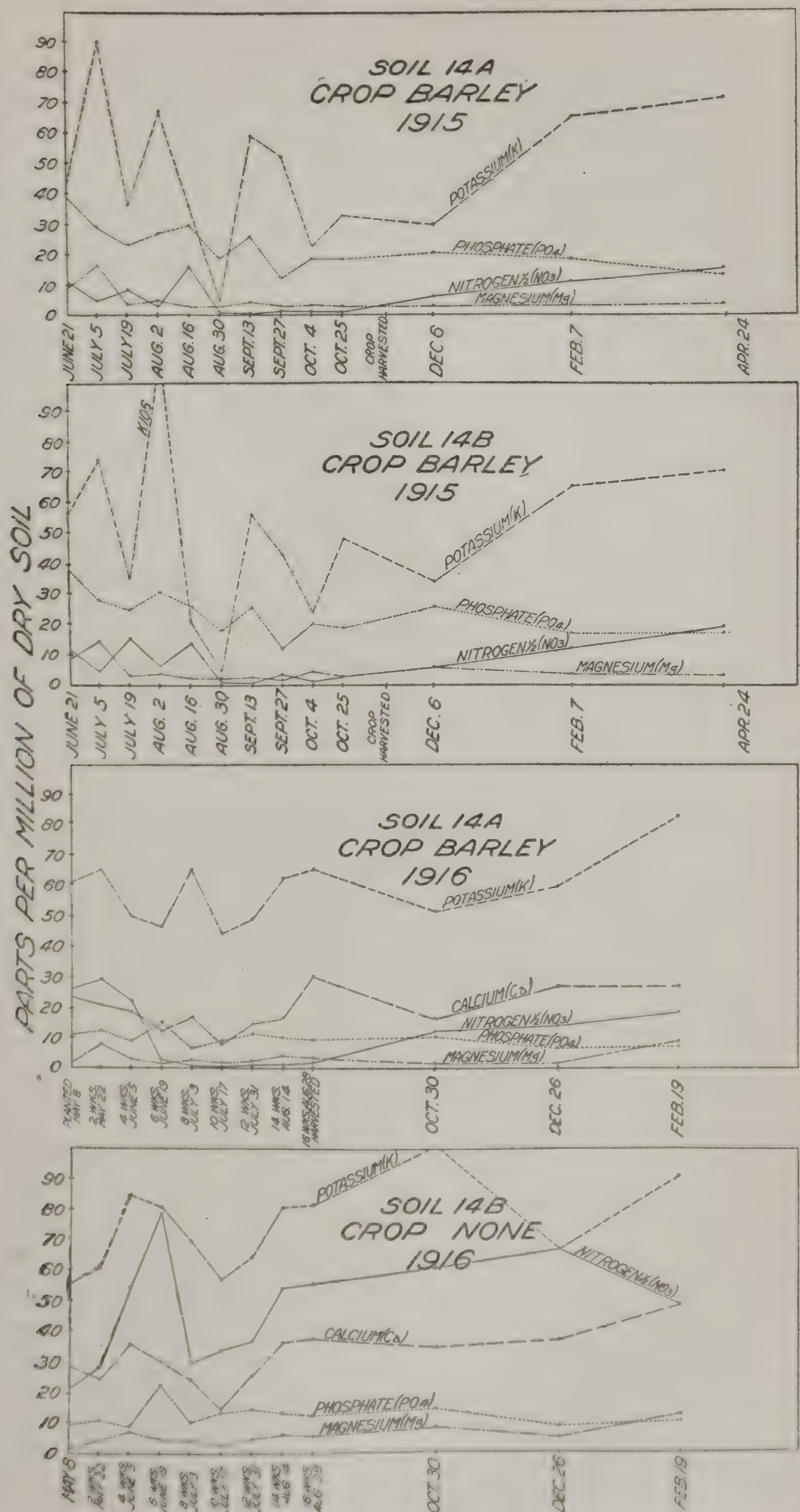

FIG. 20.—Graphs of the seasonal studies of the water extract of soil 14. Standish fine sandy loam.

SECOND SEASON, 1916

In the season of 1916 one container of each soil was again planted to the same strain of Beldi barley, and the duplicate was at all times treated in the same manner, except that no crop was grown upon it. One week before planting, the top soil to a depth of 8 or 10 inches was forked up and put in an excellent state of tilth. The soils were again sampled every two weeks from the time of planting in May until the crop was harvested in August. During the succeeding fall and winter samples were taken at approximately 8-week intervals.

The analyses of the water extracts were performed by the modified methods which have been previously outlined. During the latter portion of the sampling season cooperative work was performed by Hoagland on the samples, using the freezing-point method.

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The results of the crop yield in total crop and grain are given in Table IX. The grain yield has again been calculated to pounds and bushels per acre, and the percentage of variation from the maximum yield is shown.

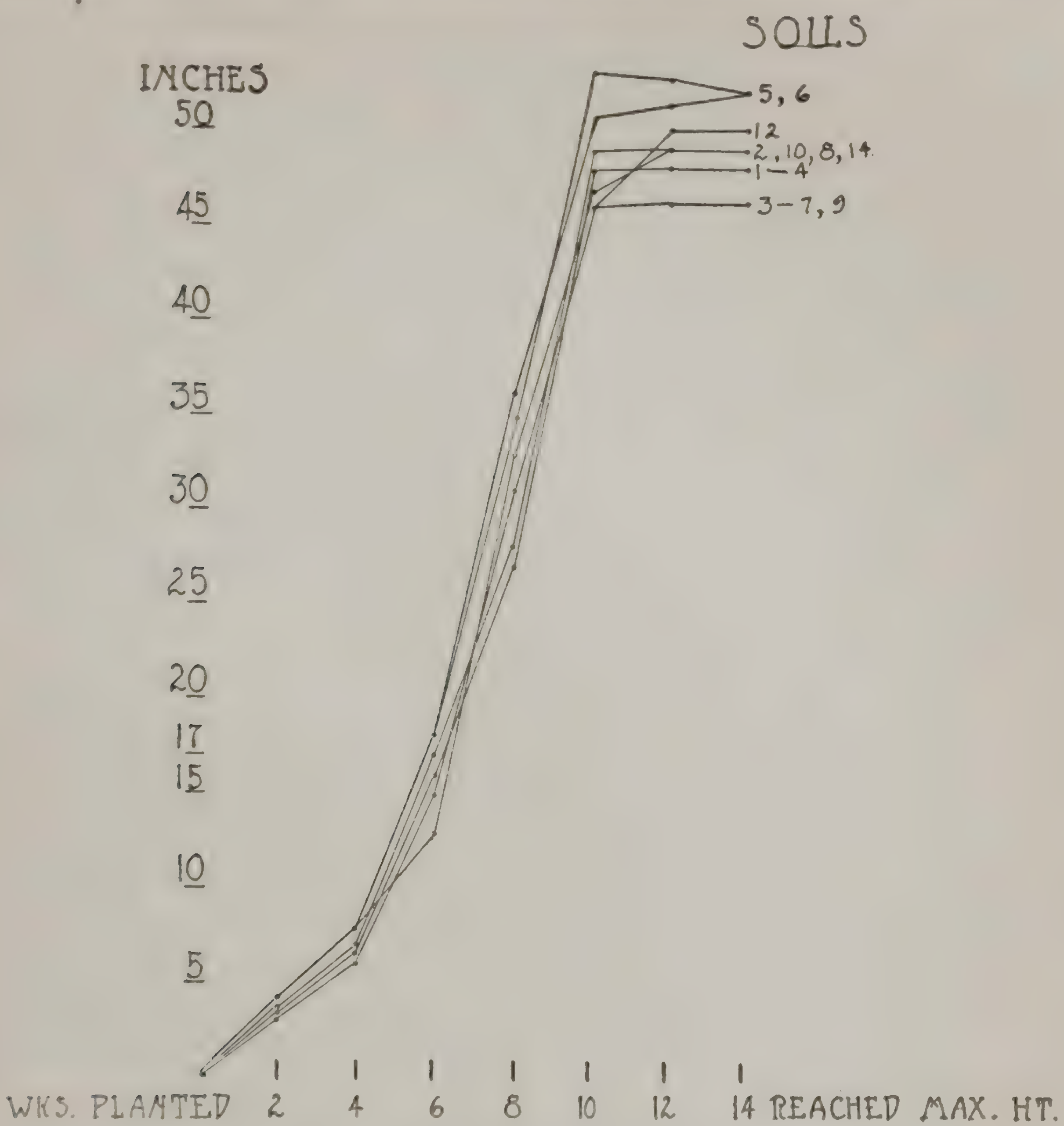

Fig. 21.—Graphs of the growth of crops in height, season of 1916.

TABLE IX.—*Crop yield in 1916*

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No. 1A	1, 474	675	5, 192	86. 5	2. 2
No. 2A	1, 530	655	5, 038	84. 0	5. 0
No. 3A	970	439	3, 377	56. 2	36. 4
No. 4A	1, 252	549	4, 223	70. 4	20. 4
No. 5A	1, 652	690	5, 307	88. 4	. 0
Yolo clay loam, 6A	1, 523	670	5, 153	85. 9	2. 8
Hanford fine sandy loam, 7A	1, 414	541	4, 161	69. 4	22. 5
Fresno fine sandy loam, 8A	1, 719	679	5, 222	87. 0	1. 6
Kimball fine sandy loam, 9A	946	357	2, 746	45. 8	48. 2
Tejunga fine sandy loam, 10A	1, 266	551	4, 238	70. 6	20. 1
Madera fine sandy loam, 11A	1, 547	625	4, 807	80. 1	9. 4
Arnold fine sandy loam, 12A	970	390	3, 000	50. 0	43. 4
Standish fine sandy loam, 14A	1, 464	630	4, 846	80. 8	8. 6

The figures obtained in the preceding season for the mean and maximum variation between duplicates will be used in order to find what will constitute a significant difference in yield. By taking the mean variation as the standard, soils 5A, 8A, 1A, 6A, and 2A are to be considered equally productive, while 11A and 14A are slightly lower. If, however, the conservative figure of the maximum variation between duplicates is used, it will be seen that No. 11A and 14A will also fall in the group of the highest yield. These relations are presented diagrammatically in figure 7.

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The soils which did not show this stimulation and liberation of nutrients were No. 3B, 9B, and 12B. These were the least productive soils of the group and also had the lowest range of soluble nutrients.

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pression, it is clear that this will not account for the differences noted. With phosphates the probable maximum is 4 to 6 per cent, while with magnesium it would vary from 6 to 10 per cent. All these corrections fail to change significantly the figures recorded.

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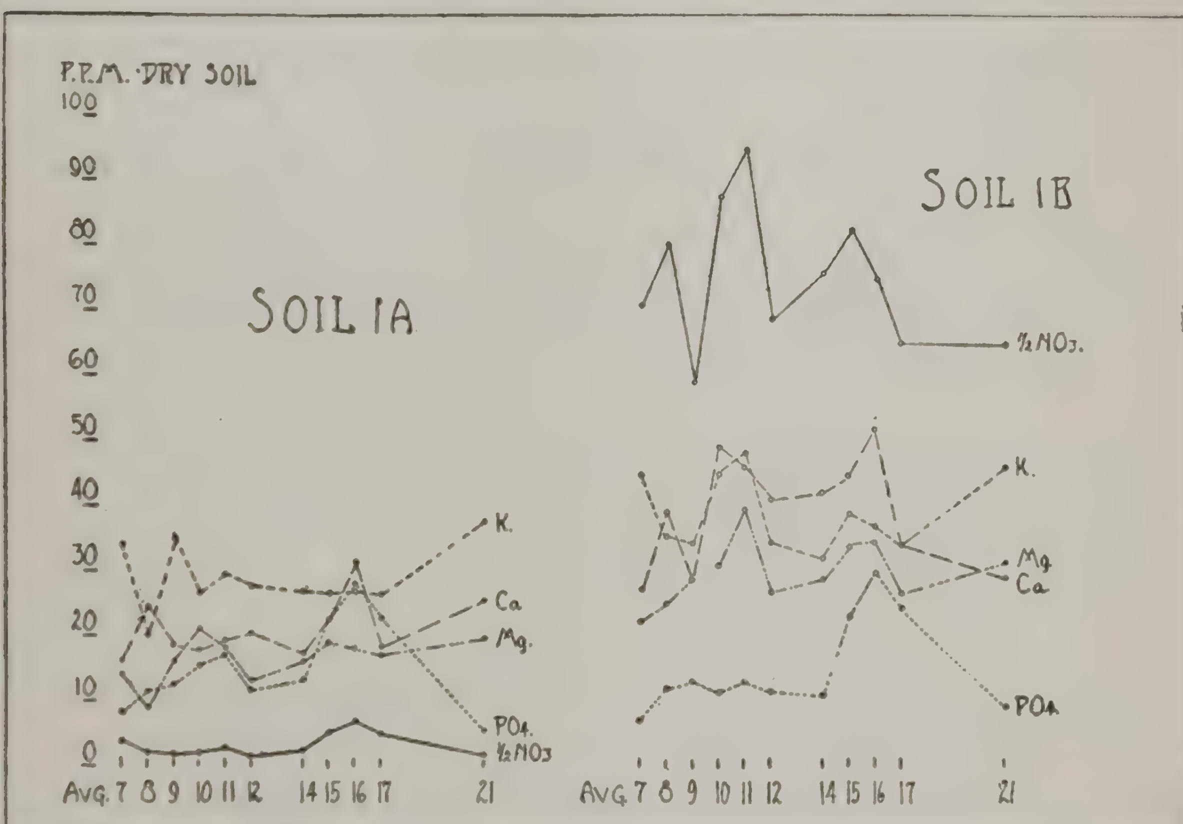

FIG. 22.—Graphs of the daily studies of the water extract of soils 1A and 1B, season of 1916.

DISCUSSION AND DEDUCTIONS

In the foregoing studies it is believed that the limitations as well as the possibilities of the experimental methods have been pointed out. It is earnestly desired that no deductions should be drawn which are not conservatively justified by the data presented.

It should be noted that the results have been obtained on 13 soils which comprise only two distinct soil types. These types are, however,

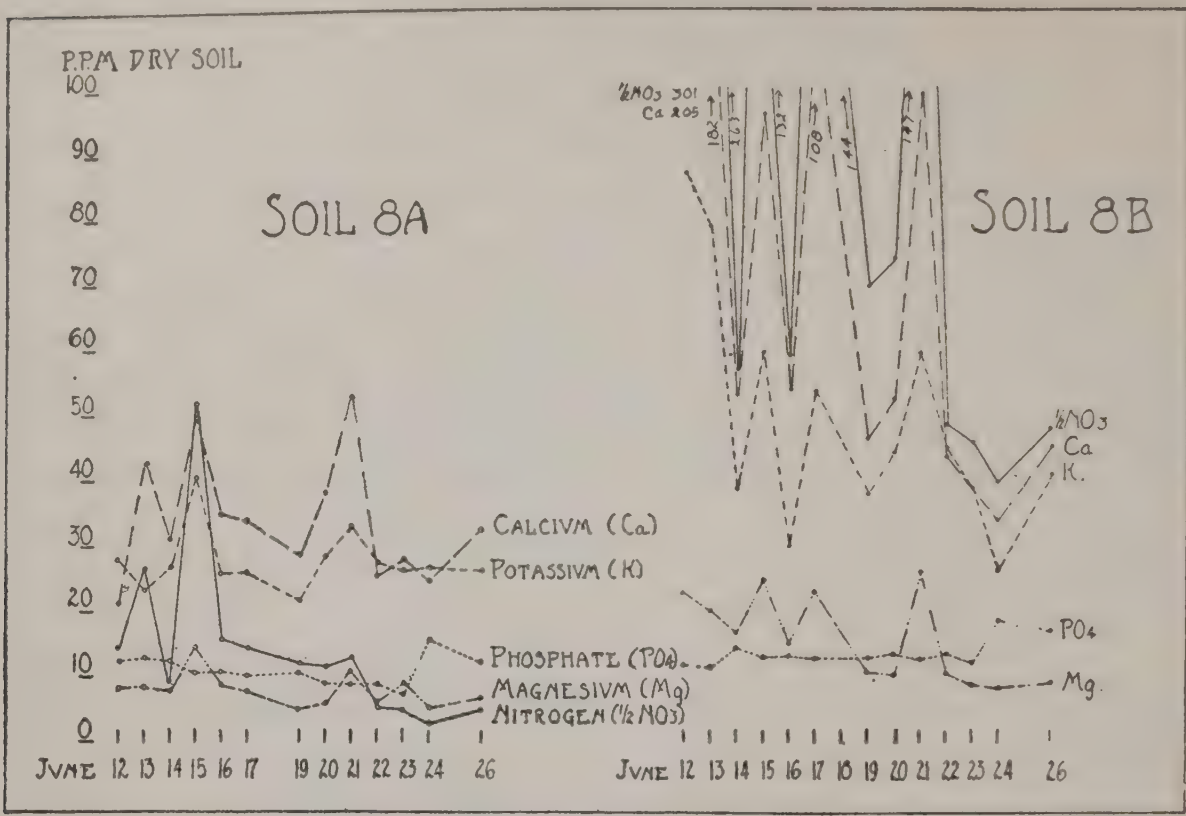

FIG. 23.—Graphs of the daily studies of the water extract of soils 8A and 8B, July, 1916.

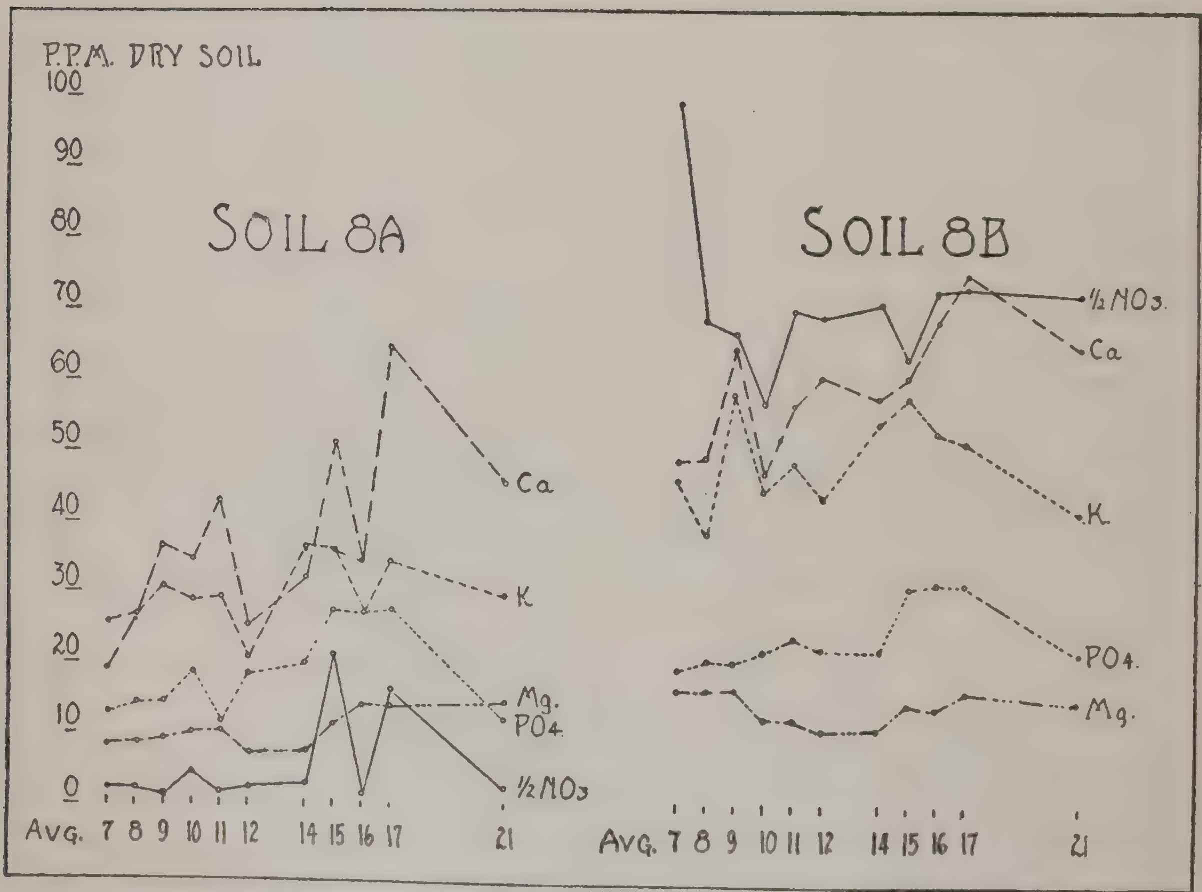

FIG. 24.—Graphs of the daily studies of the water extract of soils 8A and 8B, August, 1916.

very dissimilar in their nature; the silty clay loams are representative of decidedly complex soils with appreciable amounts of clay and other colloids, while the fine sandy loams belong to the light open soils which are common in the West. The extraction graphs yielded by both types are so similar that it is believed that comparisons can safely be made both within the types and between the two groups.

The extractions of the season of 1916 are those to which the greatest importance is attached and are those which will largely be considered in this discussion. The previous year's work brought the soils into comparable condition and also showed the probable limits of the agreement between duplicates. It is believed that the results obtained in this second season throw some light on several disputed points. The method of constant comparison between the cropped and uncropped soil is considered essential for the success of such a study. Only in this manner can any conception of the inherent capacity of a soil be obtained. When a large crop is growing on a soil, it is possible for it to affect the soluble nutrients so that the extract given by it will be equal to a moderately good or fairly poor soil.

Such a condition is seen with three fine sandy loams: No. 7, Hanford fine sandy loam, a soil of moderate production; No. 8, Fresno fine sandy loam, a very good soil; and No. 9, Kimball fine sandy loam, a soil which has produced poor crops.

The nutrients extracted from each of these three soils when cropped give practically duplicate graphs. But with the unplanted duplicate striking differences are seen. The better soils all show greater differences between the cropped and the uncropped soils than do the three poorest soils, No. 3, 9, 10, and 12. With these three it is possible that plant food is the limiting factor, but with certain soils of intermediate production, such as No. 4 and 10, it is less evident why these should not fall in the most productive group. It is claimed, however, that this method of study gives an expression of the inherent capacity of the soil to produce water-soluble plant food.

Between these three poor soils and the group of highest production there are large differences in the range of soluble nutrients. These three soils would not be classed as unfertile soils in general farming. The only one which had a small crop upon it when the original sample was collected was soil No. 12, Arnold fine sandy loam. The oats growing on this body of soil were noticeably smaller than in other portions of the field. Under the controlled conditions of the experiment the differences between these soils and the more productive must be considered significant, since they have been among the poorest soils each year. It may, therefore, be stated that among the 10 soils studied the 3 lowest in productivity also show the lowest inherent capacity to furnish soluble nutrients.

This conclusion is notably at variance with the earlier statements of Whitney and Cameron (*61*). It is believed that the experimental method which has been followed brings out the relationship between different soils with unusual clarity and effectiveness.

The influence of the crop on the soluble nutrients is another point which has been denied by the same authors. This is shown by the following quotation from Bureau of Soils, Bulletin 22:

> At the same time we have detected no constant decrease in the amount of soluble salts which could be easily detected with the methods used during the advance stages of growth of the crop, notwithstanding the considerable withdrawal there must be by the plants (*61, p. 60*).

The contrast between the curves of the planted and unplanted soils is evident at the most cursory glance. It is also clearly shown that the soil did not immediately recover and yield the same quantities of soluble nutrients as the uncropped plot, even after the crop was recovered. At the last observation recorded, on February 12, there was still an appreciable difference between the duplicate containers. In this connection it is pertinent to refer to the data obtained in a preliminary study in which a cropped soil was extracted with a diluted solution from an uncropped portion of it. In that study it was shown that this diluted solution exerted a depressing effect on the nutrients extracted from the cropped soil. This has an important application.

At the period of greatest growth of the crop, the water extract of the cropped soil approached much more closely to distilled water in the case of the planted soil than it did with the unplanted duplicate. In consequence of this fact, a greater portion of the extract obtained from it was from the solution of the actual soil minerals than was the case with the uncropped soil. The real difference between the two duplicate containers was therefore greater than the graphs would indicate. They can only be taken as the minimum difference which actually existed.

The data obtained by Hoagland, using the freezing-point method, corroborate this difference between the planted and unplanted soils. He has also observed that soils 9, Kimball fine sandy loam, and 12, Arnold fine sandy loam, are notably lower than any of the other soils in the concentration of their solutions. This coincided with the observations made from a study of the water extracts. Considering soil 3, Yolo silty clay loam, the next lowest soil in water extract, he could not draw any definite conclusion.

Jensen (*32*) in his studies on sugar beets has described a decrease in water-soluble nutrients and has also found this less noticeable in the case of phosphates. King's (*35*) figures also showed some decrease in soluble compounds as the crop advanced in growth. Harris and Butt (*24*) have observed differences in soluble salts and nitrates between cropped and fallow soils. It is believed, however, that the use of the unplanted duplicate soil and the periodic observations made upon it and the cropped

container have given a better expression of the potential power of the soil than has been obtained in the past.

The study of the unplanted soil is especially valuable because of the definite information which it furnishes in regard to the effect of fallowing and cultivation. From the results of the present investigation it can be stated that the changes occurring in water-soluble compounds, both organic and inorganic, as the result of these practices, are great and far-reaching in effect. They entirely justify the importance attached to such treatment in the past. In biennial cropping, alternating with fallowing, as practiced in California, it is probable that fully as much is gained from the increase of water-soluble nutrients as from the moisture stored up in the soil.

In the preceding studies the data obtained have been considered solely as a measure of the water-soluble nutrients obtained by a conventional procedure of extraction. From the work performed by Hoagland in collaboration the amounts extracted are definitely related to the actual soil solution. Even though the figures so obtained do indicate a range of concentrations higher than the actual truth, it has been previously pointed out that this does not alter the relationships which have been established. It is therefore believed, with this corroborative evidence, that the changes observed in the water extract reflect actual changes in the soil solution.

SUMMARY

(1) The water-soluble nutrients in 13 soils, of two different types, have been periodically determined during two seasons.

(2) Throughout the second season comparisons were made between the planted soil and its uncropped duplicate.

(3) Notable differences were observed between the nitrates, calcium, potassium, and magnesium present in the water extracts from the cropped and uncropped soils.

(4) The phosphates did not exhibit corresponding differences. Great dissimilarities were observed in the phosphate content of different soils, but in any one soil the amount was practically constant in both the cropped and uncropped plot.

(5) Striking differences occurred between the soluble nutrients present in the various uncropped soils.

(6) While the crops were growing, the concentrations of nutrients in 8 of the 13 planted soils were practically the same. These 8 included both good and poor soils.

(7) The three poorest soils yielded the smallest amounts of water-soluble nutrients and the smallest differences between the cropped and uncropped duplicates.

(8) The comparisons between the planted and unplanted duplicates furnished valuable indexes of the inherent capacities of the soils to produce nutrients.

(9) The accuracy of the methods of analysis and of the extraction procedure employed was determined, and the mean and maximum errors involved were estimated.

(10) The amounts of the water-soluble nutrients obtained by varying the ratio of soil to water were studied. The relationship of the compounds extracted did not change essentially in the lower concentrations.

(11) By comparison with freezing-point determinations the concentration of the soil solution calculated from the water extract was shown to be from two to four or five times as great as the actual soil solution.

(12) Variations in the water extract were correlated with variations in the freezing points of the same samples of soil.

(13) From the results of the freezing-point determinations it is concluded that variations in the water extract reflect actual changes in the soil solution.

(14) The results of the investigation show that large amounts of water-soluble nutrients are developed by cultivation, fallowing, and biennial cropping, and demonstrate the soundness of these practices.

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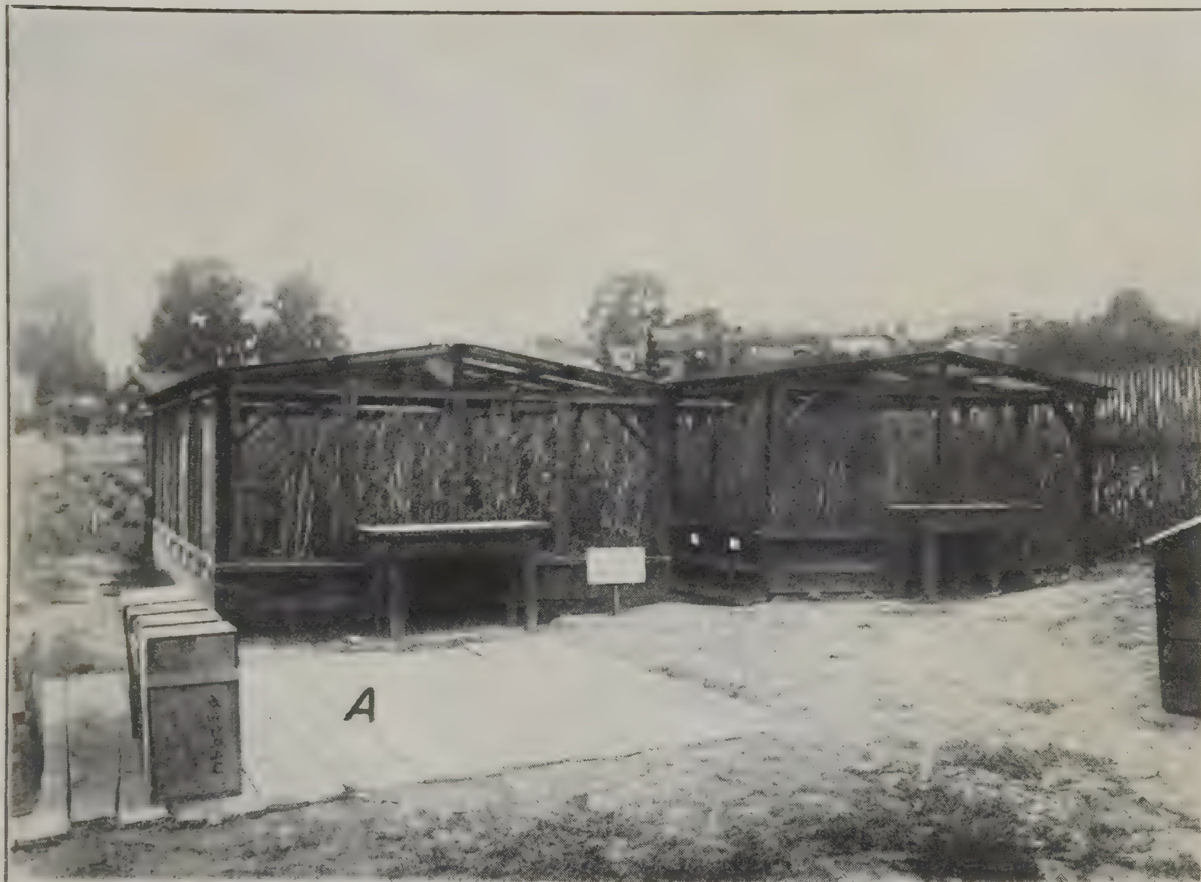
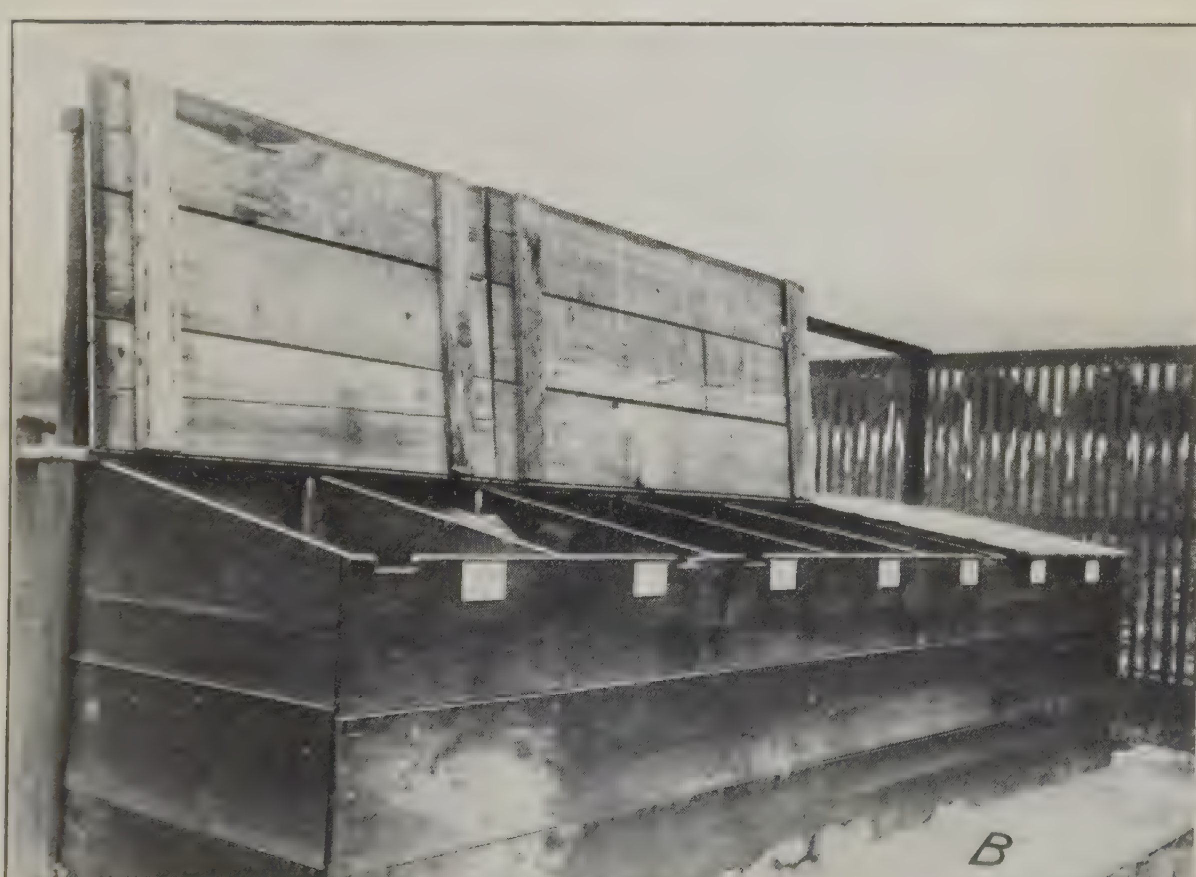
PLATE 14

A.—General views of soil containers.

B.—Bins for storage of surplus soil.





THE FREEZING-POINT METHOD AS AN INDEX OF VARIATIONS IN THE SOIL SOLUTION DUE TO SEASON AND CROP GROWTH[1]

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INTRODUCTION

In the preceding article by Stewart (*8*)[2] it has been conclusively shown that water extracts of different soils may have widely varying concentrations of important nutrient elements, and that the water-soluble substances in the 13 cropped soils were strikingly diminished in quantity when the barley crop had reached its maximum power of absorption. In the uncropped soils significant seasonal variations were also noted. These studies suggested the importance of correlating the water extracts with the actual soil solution, the immediate source of nutrients for the plant.

Various attempts have been made to separate the soil solution, but in no case has any appreciable quantity of solution been obtained when a soil contained only the optimum percentage of moisture. Recently Bouyoucos and McCool (*2*) have proposed a method which seems capable of giving direct experimental evidence concerning the concentration of the soil solution. The procedure consists in determining the depression of the freezing point in the soil itself under varying moisture conditions. The soils used in the investigation described by Stewart seemed uniquely adapted for further study by the freezing-point method. Accordingly such determinations were made on the various soils under controlled conditions, and the present paper deals with observations made on the depressions of the freezing point in soils as affected by season, cropping, moisture content, and other factors of significance in plant growth.

GENERAL METHOD OF PROCEDURE

The technic employed was essentially that described by Bouyoucos and McCool (*2*). The experience of the Agricultural Chemistry Laboratory confirms their statements with regard to the possibility of obtaining closely agreeing duplicates and general consistency of results. The freezing-point blank with distilled water, however, was not found to maintain a constant value from day to day, and to obviate any possible

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[2] Reference is made by number (italic) to "Literature cited," p. 394-395.

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error from this source, determinations on pure water were checked several times each day. The general magnitude of error in technic is indicated by the following typical instances of duplicate experiments:

Soil No.	Freezing-point depression.	Soil No.	Freezing-point depression.
	°C.		°C.
10A	0.065	15A	0.052
10A	.062	14A	.043
1B	.066	14A	.050
1B	.069	4B	.157
15A	.049	4B	.155

DESCRIPTION OF SOILS USED

In the article by Stewart (*8*) the reader will find a detailed description of the soils under the same laboratory numbers. For the sake of convenient reference, a list of the soils used is included here (Table I). Each soil was investigated under three general conditions: (1) Soil from tanks in which barley crop was grown for two seasons; (2) corresponding soils kept under identical conditions but with no crop the second season; (3) soils air-dried, sifted, and kept two years in closed bins. All tank soils were kept constantly at nearly optimum moisture contents with distilled water.

TABLE I.—*Description of soils used in this investigation*

Laboratory No.	Soil series and type.	Origin.
1, 2, 3	Yolo silty clay loam	Sacramento Valley.
4, 5, 6	do	Santa Clara Valley.
7	Hanford fine sandy loam	Southern California.
8	Fresno fine sandy loam	San Joaquin Valley.
9	Kimball fine sandy loam	Southern California.
10	Tejunga fine sandy loam	Do.
11	Madera fine sandy loam	San Joaquin Valley.
12	Arnold fine sandy loam	Do.
14	Standish fine sandy loam	Virgin desert soil.

UNFREE WATER OF SOILS

One of the most important factors affecting the concentration of the soil solution is the moisture content. This has already been pointed out by Bouyoucos and McCool, and the former has recently devised a dilatometer method for more accurately measuring the unfree water of a soil (*1*). In the present investigation an attempt was made to approximate the unfree water in each soil by careful determinations of the freezing-point depressions at different moisture contents. These data could then be used in reducing the observed depressions to definite and comparable moisture percentages, especially where the observed

moisture contents differed only slightly from the standard. It has been shown by Bouyoucos and McCool that freezing-point depressions vary with the water content of the soil, not usually in direct ratio, but in such manner as to necessitate the assumption that a certain fraction of the total water present is so combined that it does not form an effective part of the soil solution and is not subject to freezing. The percentage of combined water would vary greatly with the type of soil, clay soils having a large proportion of their water in the unfree state. The above considerations, advanced by Bouyoucos and McCool, have been made the basis for the determinations of unfree water in the soils used in this investigation. Portions of each soil were divided into two samples the moisture contents of which were so adjusted as to give a difference of about 5 per cent. Each sample was thoroughly mixed and kept overnight in a tight jar. Careful determinations of total moisture and freezing-point depression were then made. From these data it is possible to calculate what proportion of the moisture must be subtracted from the total in order that the percentages of free water may give the same ratios as the freezing-point depressions. Such estimates may not have a high degree of accuracy, but it is probably sufficient for the purposes in hand. Table II presents the results for the unfree water in each soil.

TABLE II.—*Estimation of unfree water in soils*

Soil No.	Moisture.	Freezing-point depression.	Unfree water.	Soil No.	Moisture.	Freezing-point depression.	Unfree water.
	Per cent.	° *C.*	*Per cent.*		*Per cent.*	° *C.*	*Per cent.*
1C	20.0	0.098	18.0	8C	16.6	0.215	4.0
1C	22.4	.034		8C	8.1	.655	
2C	18.0	.140	13.0	9C	12.8	.099	6.0
2C	23.0	.069		9C	17.2	.059	
3C	18.4	.357	14.0	10C	12.8	.153	8.0
3C	24.0	.149		10C	17.8	.074	
4C	19.6	.362	13.0	11C	13.2	.331	6.0
4C	23.6	.210		11C	18.8	.197	
5C	18.8	.089	13.0	12C	13.2	.130	4.0
5C	23.2	.050		12C	18.0	.089	
6C	19.6	.282	17.7	14C	14.0	.254	7.5
6C	24.4	.079		14C	18.4	.149	
7C	12.4	.284	6.0				
7C	17.6	.167					

SEASONAL VARIATIONS IN CROPPED AND IN UNCROPPED SOILS

One of the primary objects of this investigation was to follow through the season the concentrations of the soil solution in each soil and to compare at each period of sampling the cropped soils with their uncropped duplicates. The first determinations of freezing-point depressions were made early in July, 1916, and at intervals from that date until the following May. The barley crop was planted in May, 1916, and harvested

in August. The samples of soil used in this work were identical with those employed by Stewart in making water extractions. In general, the moisture contents of the uncropped and cropped soils were very similar, but it was not always feasible to keep them in absolute agreement. The data are therefore presented in two forms: First, as a table showing the observed depressions and actual percentages of total moisture present, together with the corresponding osmotic pressures and parts per million of total solids in solution, as calculated by the methods of

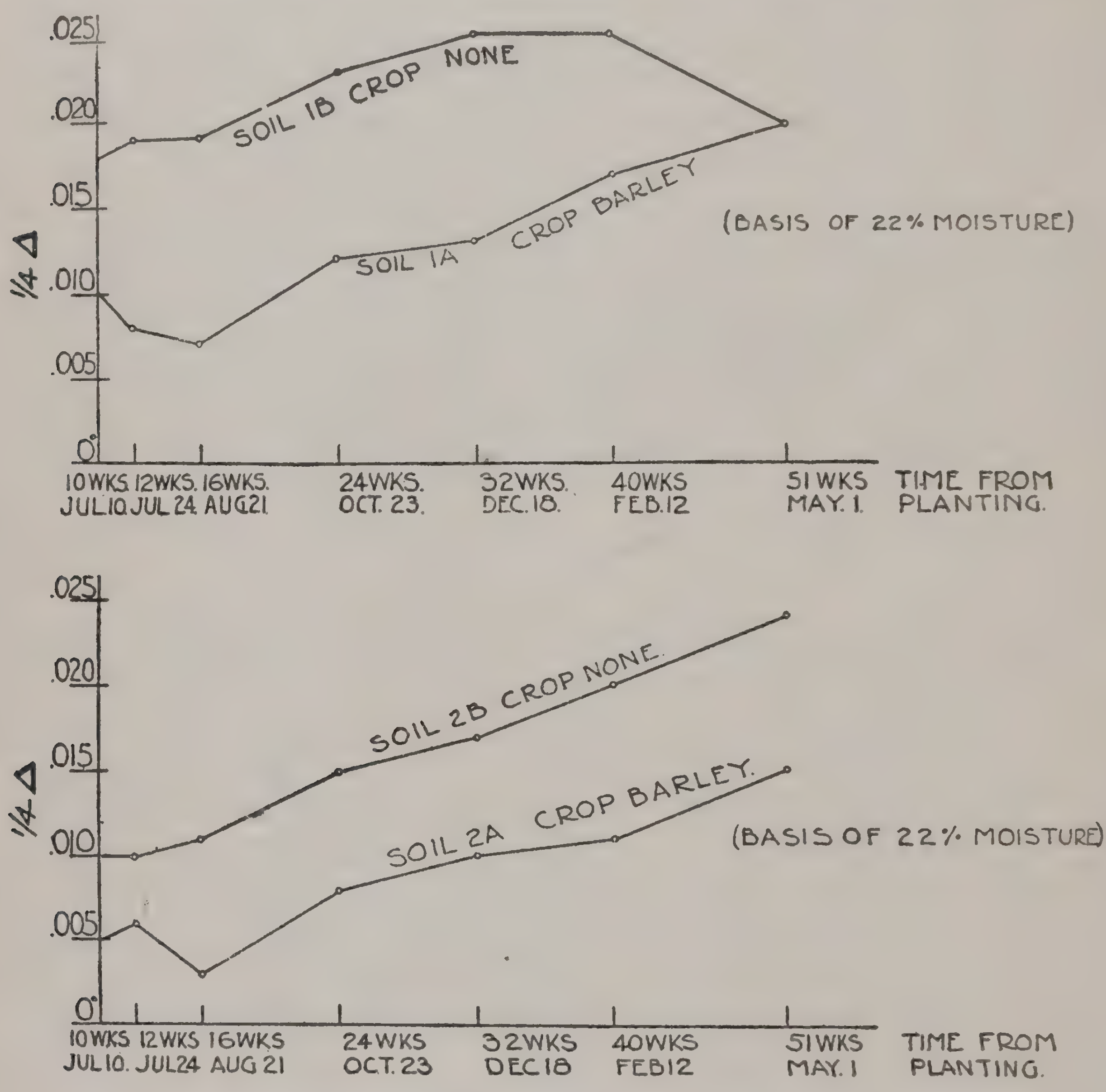

FIG. 1.—Graphs of the depressions of the freezing point in soils 1 and 2, with and without crop.

Bouyoucos and McCool. In the second place, the observed depressions have been calculated to uniform moisture contents, 17 per cent for all of the fine sandy loams and 22 per cent for the silty clay loams. In making these estimates the percentages of unfree water already presented have been used. The corrected depressions have been plotted for each pair of soils. In this way the cropped and the uncropped soils, as well as the different soils in each group, may be compared readily (fig. 1–8). (Cf. Table III.)

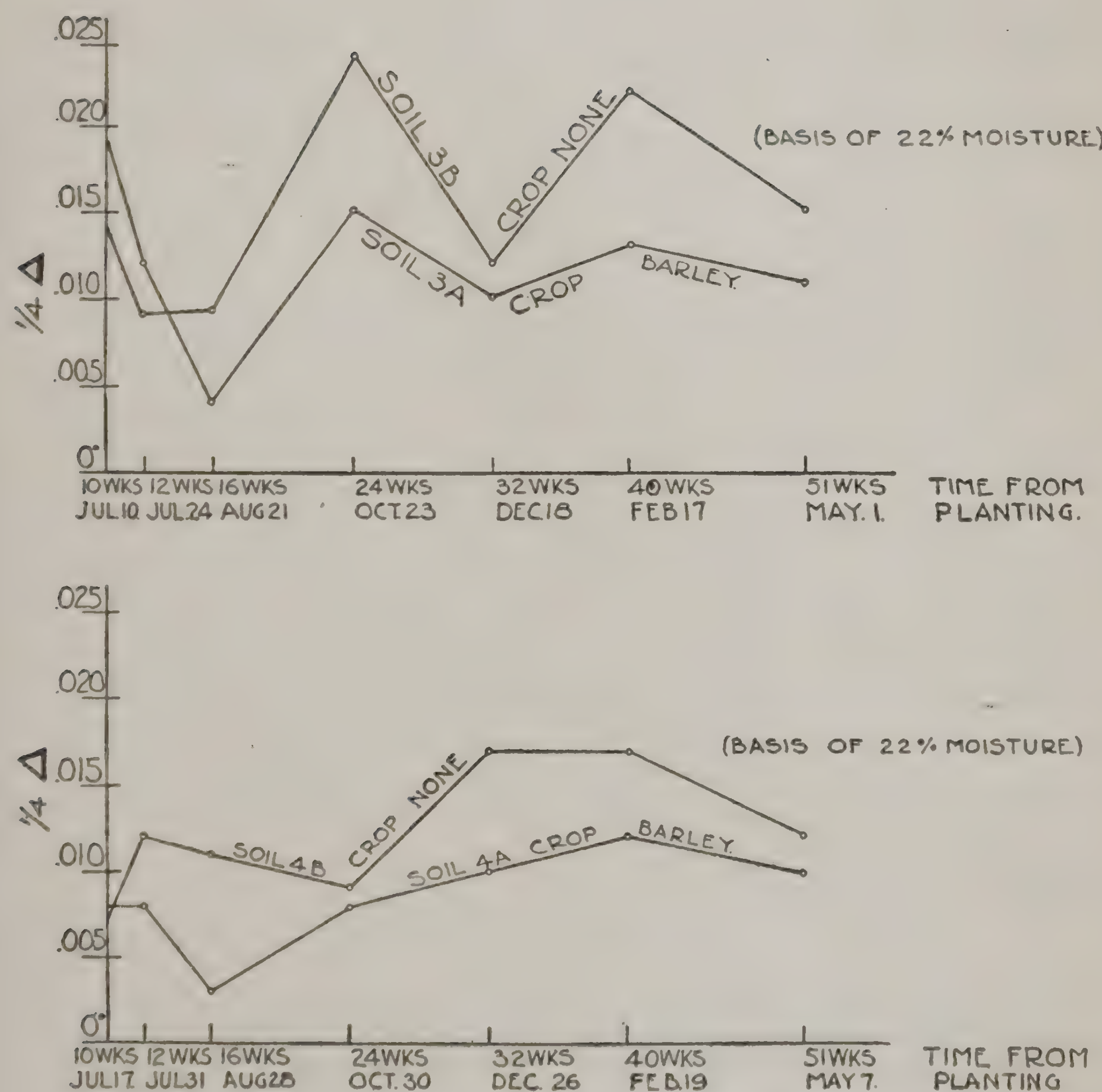

FIG. 2.—Graphs of the depressions of the freezing point in soils 3 and 4, with and without crop.

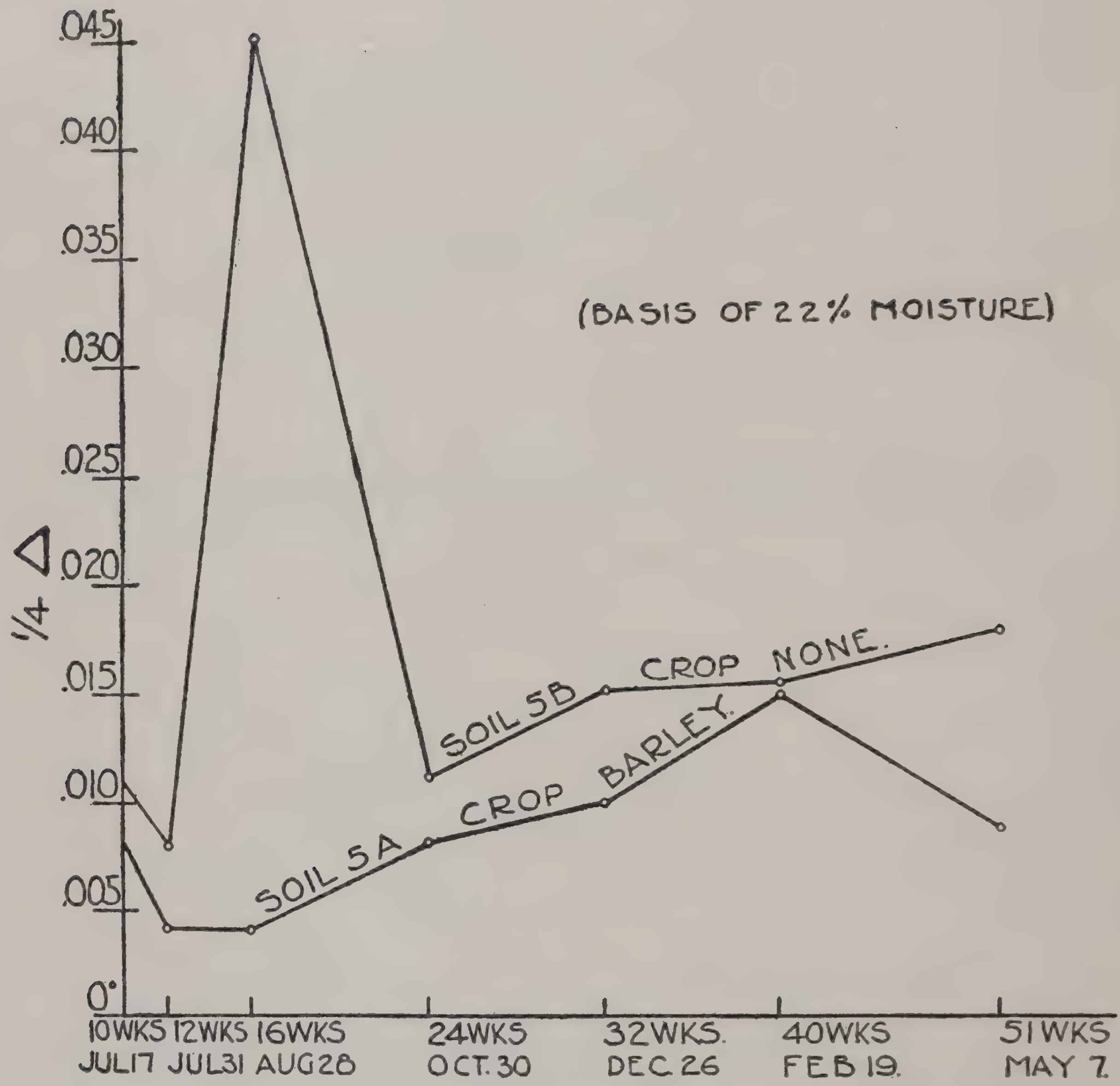

FIG. 3.—Graphs of the depressions of the freezing point in soil 5, with and without crop.

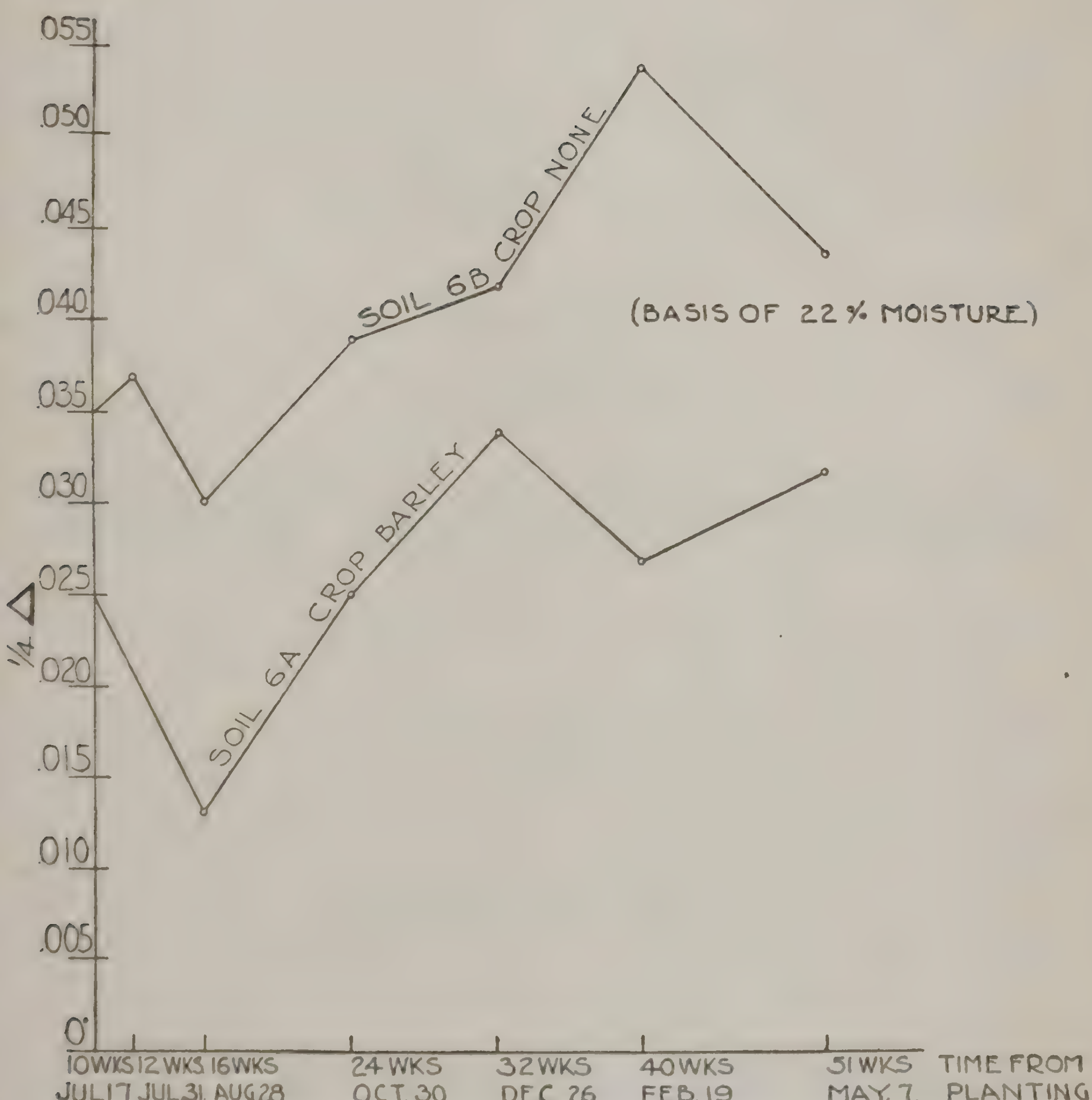

Fig. 4.—Graphs of the depressions of the freezing point in soil 6, with and without crop.

27809°—18——6

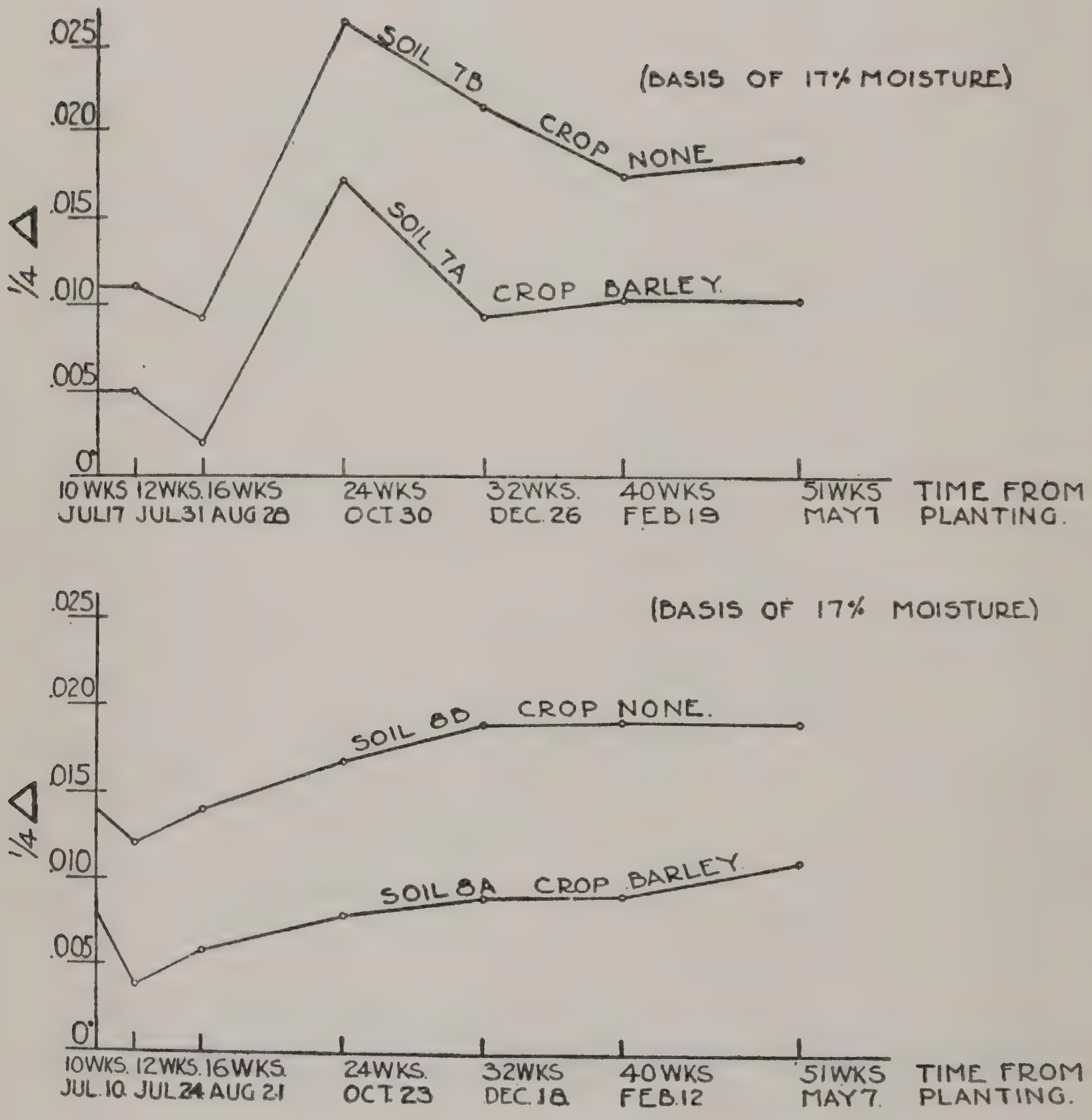

Fig. 5.—Graphs of the depressions of the freezing point in soils 7 and 8, with and without crop.

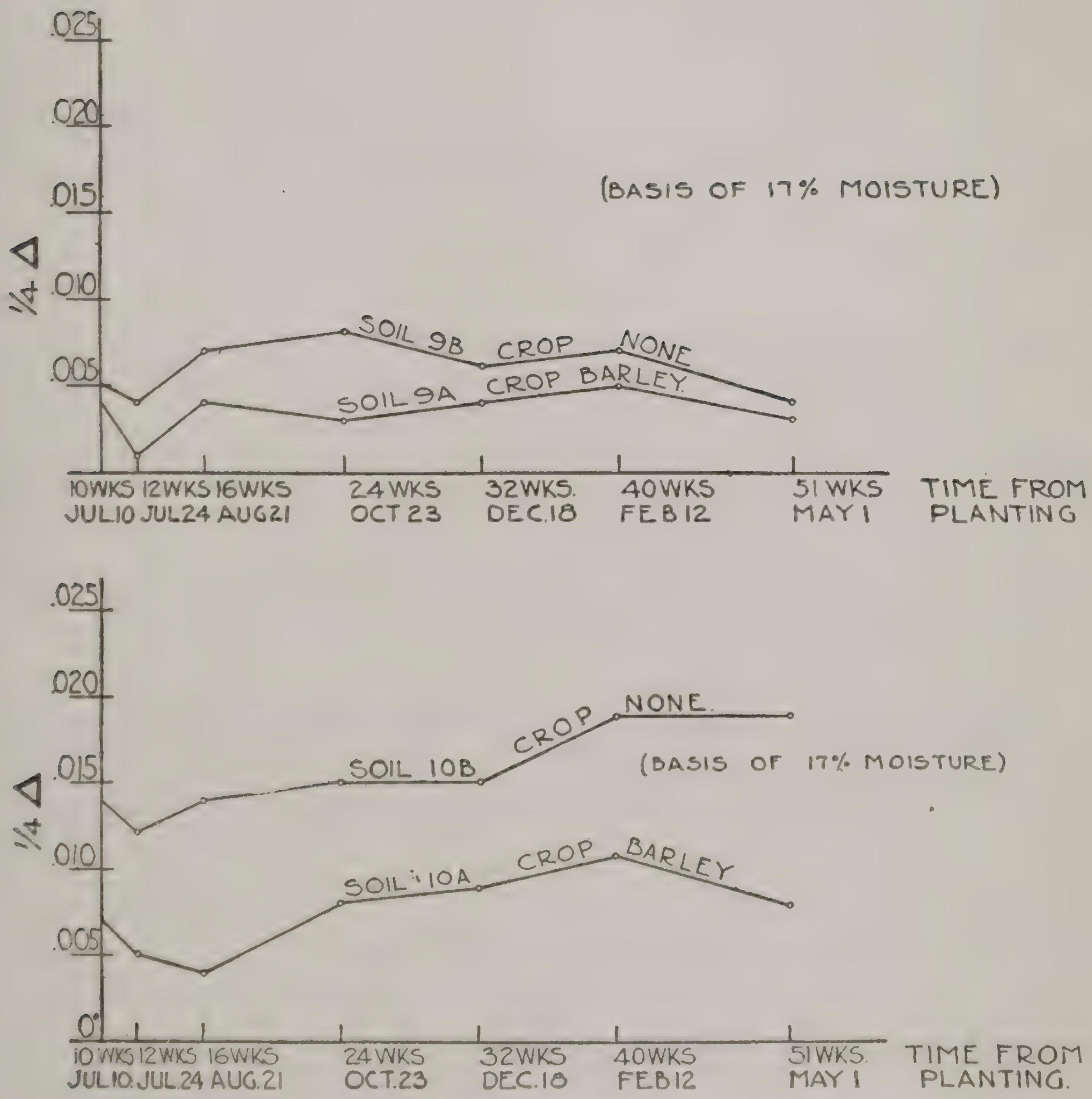

FIG. 6.—Graphs of the depressions of the freezing point in soils 9 and 10, with and without crop.

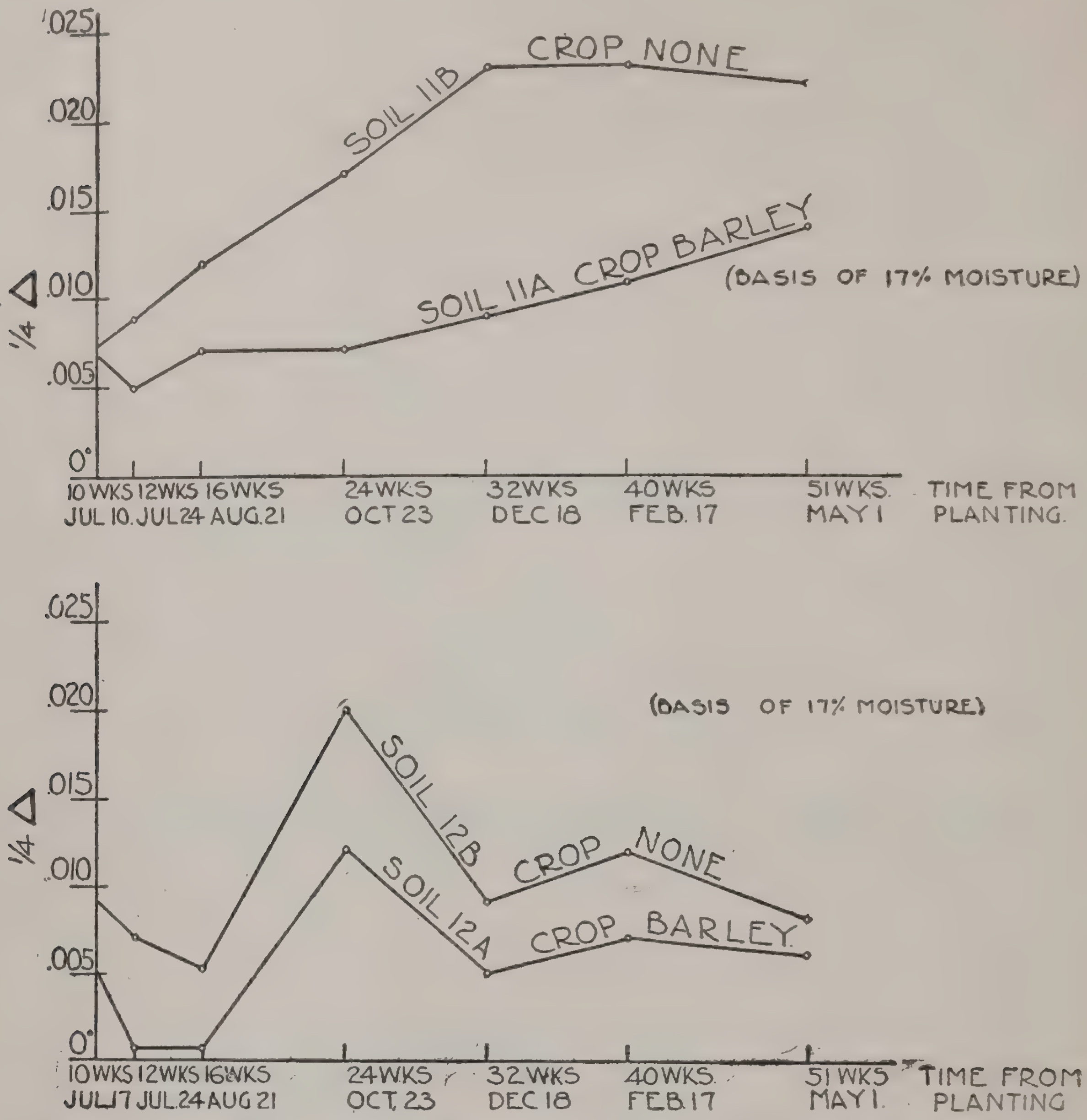

FIG. 7.—Graphs of the depressions of the freezing point in soils 11 and 12, with and without crop.

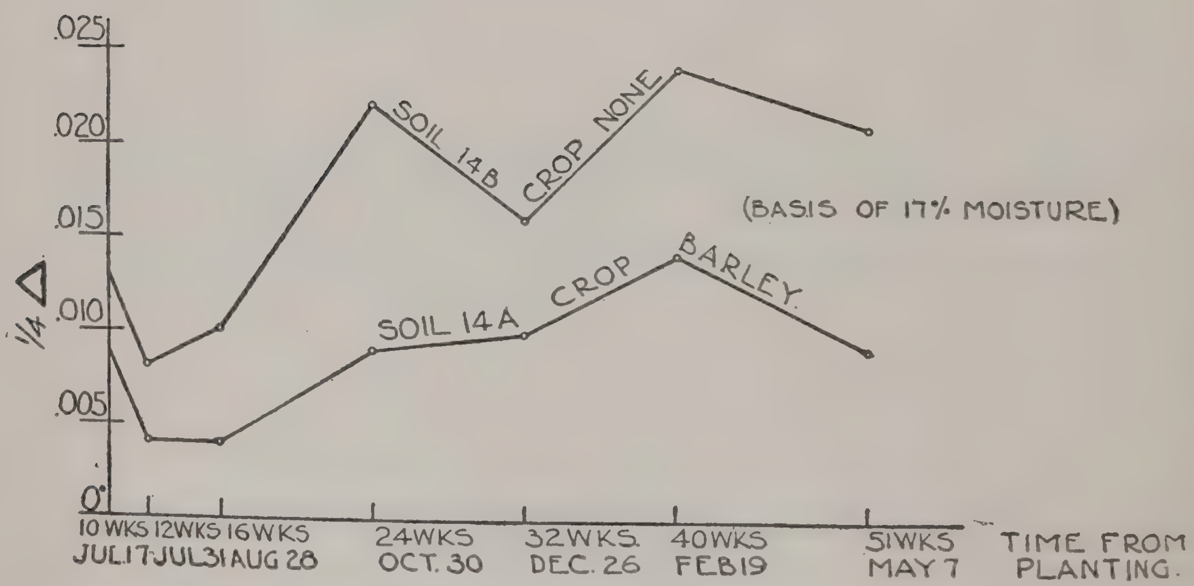

FIG. 8.—Graphs of the depressions of the freezing point in soil 14, with and without crop.

TABLE III.—*Observed and calculated freezing-point depressions*

Date.	Moisture.		Observed freezing-point depression.		Osmotic pressure (atmospheres).		Calculated total solids in soil solution.		Freezing-point depression calculated to uniform moisture.[a]	
	Soil 1A.	Soil 1B.	Soil 1A.	Soil 1B.	Soil 1A.	Soil 1B.	Soil 1A.	Soil 1B.	Soil 1A.	Soil 1B.
	P. ct.	*P. ct.*	*° C.*	*° C.*			*P. p. m.*	*P. p. m.*	*° C.*	*° C.*
July 10	23.2	22.4	0.030	0.064	0.36	0.77	900	2,000	0.039	0.070
July 24	22.5	22.7	.029	.065	.35	.78	900	2,000	.033	.076
Aug. 21	23.9	23.0	.019	.062	.23	.74	600	1,900	.028	.77
Oct. 23	21.8	21.1	.050	.118	.60	1.43	1,600	3,700	.047	.091
Dec. 18	23.4	22.3	.037	.095	.44	1.15	1,200	3,000	.050	.102
Feb. 12	22.7	21.7	.056	.108	.68	1.31	1,800	3,400	.066	.100
May 1	21.9	20.3	.082	.138	.99	1.56	2,600	4,300	.080	.079
	Soil 2A.	Soil 2B.	Soil 2A.	Soil 2B.	Soil 2A.	Soil 2B.	Soil 2A.	Soil 2B.	Soil 2A.	Soil 2B.
July 10	20.4	21.4	0.026	0.044	0.32	0.53	800	1,400	0.021	0.041
July 24	19.7	20.8	.034	.047	.41	.56	1,100	1,500	.025	.041
Aug. 21	23.7	21.3	.008	.049	.10	.59	300	1,500	.010	.045
Oct. 23	18.3	18.9	.055	.092	.66	1.11	1,700	2,900	.032	.060
Dec. 18	20.4	20.0	.047	.087	.56	1.05	1,500	2,700	.039	.068
Feb. 12	20.2	20.2	.056	.101	.68	1.22	1,800	3,200	.045	.081
May 1	17.4	17.3	.131	.201	1.47	2.42	4,100	6,300	.064	.096
	Soil 3A.	Soil 3B.	Soil 3A.	Soil 3B.	Soil 3A.	Soil 3B.	Soil 3A.	Soil 3B.	Soil 3A.	Soil 3B.
July 10	18.6	22.2	0.130	0.055	1.46	0.66	4,100	1,700	0.075	0.056
July 24	19.0	22.2	.078	.036	.94	.44	2,400	1,100	.049	.037
Aug. 21	22.0	22.7	.017	.034	.20	.41	500	1,100	.017	.037
Oct. 23	20.5	20.9	.075	.110	.90	1.33	2,300	3,400	.061	.095
Dec. 18	20.9	20.7	.048	.060	.58	.72	1,500	1,900	.041	.050
Feb. 12	20.6	19.9	.062	.120	.74	1.45	1,900	3,800	.051	.089
May 1	19.1	17.5	.068	.135	.82	1.52	2,100	4,200	.043	.059
	Soil 4A.	Soil 4B.	Soil 4A.	Soil 4B.	Soil 4A.	Soil 4B.	Soil 4A.	Soil 4B.	Soil 4A.	Soil 4B.
July 17	18.8	18.5	0.048	0.047	0.58	0.56	1,500	1,500	0.031	0.029
July 31	18.6	19.6	.050	.066	.60	.80	1,600	2,100	.031	.048
Aug. 28	19.2	20.0	.017	.057	.20	.68	500	1,800	.012	.044
Oct. 30	17.3	16.7	.068	.082	.82	.99	2,100	2,600	.033	.034
Dec. 26	19.3	18.0	.055	.121	.66	1.46	1,700	3,800	.038	.067
Feb. 19	17.6	17.2	.098	.148	1.19	1.179	3,100	4,600	.050	.069
May 7	16.5	15.8	.106	.157	1.29	1.89	3,300	4,900	.041	.049

[a] Soils 1, 2, 3, 4, 5, and 6 calculated to 22 per cent of moisture. Soils 7, 8, 9, 10, 11, 12, and 14 calculated to 17 per cent of moisture.

TABLE III.—*Observed and calculated freezing-point depressions*—Continued

Date.	Moisture.		Observed freezing-point depression.		Osmotic pressure (atmospheres).		Calculated total solids in soil solution.		Freezing-point depression calculated to uniform moisture.	
	Soil 5A.	Soil 5B.	Soil 5A.	Soil 5B.	Soil 5A.	Soil 5B.	Soil 5A.	Soil 5B.	Soil 5A.	Soil 5B.
	P. ct.	*P. ct.*	*° C.*	*° C.*			*P. p. m.*	*P. p. m.*	*° C.*	*° C.*
July 17	16. 2	18. 7	0. 093	0. 069	1. 13	0. 83	2, 900	2, 200	0. 033	0. 044
July 31	18. 4	20. 6	. 029	. 039	. 35	. 47	900	1, 200	. 017	. 033
Aug. 28	21. 9	21. 1	. 017	. 157	. 20	1. 89	500	4, 900	. 017	. 141
Oct. 30	16. 8	17. 5	. 079	. 088	. 95	1. 07	2, 500	2, 800	. 033	. 044
Dec. 26	18. 2	18. 0	. 066	. 106	. 80	1. 29	2, 100	3, 300	. 038	. 059
Feb. 19	18. 1	17. 2	. 106	. 115	1. 29	1. 39	3, 300	3, 600	. 060	. 054
May 7	16. 9	19. 2	. 079	. 101	. 95	1. 22	2, 500	3, 200	. 034	. 070
	Soil 6A.	Soil 6B.	Soil 6A.	Soil 6B.	Soil 6A.	Soil 6B.	Soil 6A.	Soil 6B.	Soil 6A.	Soil 6B.
July 17	18. 8	23. 8	0. 399	0. 097	4. 81	1. 17	12, 000	3, 000	0. 102	0. 138
July 31	......	25. 2	......	. 086	......	1. 05	....	2, 700	......	. 150
Aug. 28	23. 4	24. 9	. 040	. 072	. 48	. 86	1, 300	2, 300	. 053	. 121
Oct. 30	24. 1	24. 4	. 068	. 101	. 82	1. 22	2, 100	3, 200	. 101	. 157
Dec. 26	24. 5	24. 8	. 085	. 103	1. 02	1. 25	2, 700	3, 200	. 135	. 170
Feb. 19	24. 8	25. 0	. 066	. 126	. 80	1. 52	2, 100	3, 900	. 109	. 214
May 7	23. 0	22. 6	. 104	. 153	1. 26	1. 85	3, 300	4, 800	. 128	. 174
	Soil 7A.	Soil 7B.	Soil 7A.	Soil 7B.	Soil 7A.	Soil 7B.	Soil 7A.	Soil 7B.	Soil 7A.	Soil 7B.
July 17	13. 7	15. 9	0. 028	0. 048	0. 34	0. 58	900	1, 500	0. 020	0. 043
July 31	13. 6	16. 9	. 026	. 042	. 32	. 50	800	1, 300	. 018	. 042
Aug. 28	19. 3	16. 6	. 007	. 035	. 08	. 42	200	1, 100	. 009	. 034
Oct. 30	14. 9	13. 6	. 082	. 151	. 99	1. 82	2, 600	4, 700	. 066	. 104
Dec. 26	15. 9	14. 7	. 038	. 108	. 46	1. 31	1, 200	3, 400	. 034	. 085
Feb. 19	14. 0	14. 0	. 053	. 093	. 64	1. 13	1, 700	2, 900	. 041	. 068
May 7	12. 5	12. 8	. 070	. 116	. 84	1. 41	2, 200	3, 600	. 041	. 072
	Soil 8A.	Soil 8B.	Soil 8A.	Soil 8B.	Soil 8A.	Soil 8B.	Soil 8A.	Soil 8B.	Soil 8A.	Soil 8B.
July 10	13. 2	14. 4	0. 038	0. 064	0. 46	0. 77	1, 200	2, 000	0. 027	0. 051
July 24	14. 1	14. 8	. 015	. 053	. 18	. 64	500	1, 700	. 012	. 044
Aug. 21	15. 9	15. 9	. 021	. 056	. 25	. 68	700	1, 800	. 019	. 051
Oct. 23	9. 9	10. 0	. 060	. 138	. 72	1. 56	1, 900	4, 300	. 027	. 064
Dec. 18	12. 7	12. 5	. 049	. 111	. 59	1. 34	1, 500	3, 400	. 033	. 073
Feb. 12	10. 6	10. 8	. 062	. 135	. 74	1. 52	1, 900	4, 200	. 032	. 071
May 7	8. 2	7. 9	. 122	. 214	1. 47	2. 58	3, 800	6, 700	. 039	. 071
	Soil 9A.	Soil 9B.	Soil 9A.	Soil 9B.	Soil 9A.	Soil 9B.	Soil 9A.	Soil 9B.	Soil 9A.	Soil 9B.
July 10	14. 3	13. 4	0. 018	0. 034	0. 22	0. 41	600	1, 100	0. 014	0. 023
July 24	14. 0	13. 2	. 007	. 026	. 08	. 32	200	800	. 005	. 017
Aug. 21	16. 9	13. 1	. 014	. 042	. 17	. 50	400	1, 300	. 014	. 027
Oct. 23	10. 7	9. 7	. 028	. 092	. 34	1. 11	900	2, 900	. 012	. 031
Dec. 18	11. 0	10. 6	. 031	. 058	. 37	. 70	1, 000	1, 800	. 014	. 024
Feb. 12	9. 4	9. 5	. 057	. 087	. 68	1. 05	1, 800	2, 700	. 018	. 028
May 1	7. 1	6. 9	. 118	. 200	1. 43	2. 41	3, 700	6, 300	. 012	. 016

TABLE III.—*Observed and calculated freezing-point depressions*—Continued

Date.	Moisture.		Observed freezing-point depression.		Osmotic pressure (atmospheres).		Calculated total solids in soil solution.		Freezing-point depression calculated to uniform moisture.	
	Soil 10A.	Soil 10B.	Soil 10A.	Soil 10B.	Soil 10A.	Soil 10B.	Soil 10A.	Soil 10B.	Soil 10A.	Soil 10B.
	P. ct.	*P. ct.*	° *C.*	° *C.*			*P. p. m.*	*P. p. m.*	° *C.*	° *C.*
July 10	15.7	16.6	0.037	0.056	0.44	0.67	1,200	1,800	0.028	0.054
July 24	16.0	16.1	.023	.051	.28	.61	700	1,600	.021	.047
Aug. 21	19.6	17.0	.012	.057	.14	.68	400	1,800	.015	.057
Oct. 23	14.9	13.6	.041	.099	.49	1.19	1,300	3,100	.033	.061
Dec. 18	15.1	14.6	.046	.079	.55	.95	1,400	2,500	.036	.058
Feb. 12	14.1	13.4	.066	.125	.79	1.51	2,100	3,900	.045	.075
May 1	11.5	11.0	.094	.228	1.14	2.75	2,900	7,100	.033	.076
	Soil 11A.	Soil 11B.	Soil 11A.	Soil 11B.	Soil 11A.	Soil 11B.	Soil 11A.	Soil 11B.	Soil 11A.	Soil 11B.
July 10	14.9	11.2	0.034	0.060	0.41	0.72	1,100	1,900	0.028	0.028
July 24	12.7	15.8	.035	.041	.42	.49	1,100	1,300	.021	.037
Aug. 21	16.6	16.3	.027	.052	.32	.62	800	1,600	.026	.049
Oct. 23	13.9	13.2	.037	.101	.44	1.22	1,200	3,200	.027	.066
Dec. 18	14.5	13.6	.047	.134	.56	1.51	1,500	4,200	.036	.093
Feb. 17	13.3	13.5	.065	.133	.78	1.50	2,000	4,200	.043	.091
May 1	11.0	10.7	.121	.203	1.47	2.45	3,800	6,300	.055	.087
	Soil 12A.	Soil 12B.	Soil 12A.	Soil 12B.	Soil 12A.	Soil 12B.	Soil 12A.	Soil 12B.	Soil 12A.	Soil 12B.
July 17	11.1	12.3	0.033	0.054	0.40	0.65	1,000	1,700	0.018	0.034
July 24	14.4	13.5	.001	.036	.01	.44	50	1,100	.001	.026
Aug. 21	16.3	14.5	.003	.026	.04	.32	100	800	.003	.021
Oct. 23	14.4	11.4	.061	.138	.73	1.56	1,900	4,300	.049	.079
Dec. 18	13.9	11.7	.030	.059	.36	.71	900	1,800	.023	.035
Feb. 17	12.7	11.4	.043	.083	.52	1.01	1,300	2,600	.029	.047
May 1	10.0	9.6	.048	.077	.58	.92	1,500	2,400	.022	.033
	Soil 14A.	Soil 14B.	Soil 14A.	Soil 14B.	Soil 14A.	Soil 14B.	Soil 14A.	Soil 14B.	Soil 14A.	Soil 14B.
July 17	15.7	18.4	0.041	0.045	0.49	0.54	1,300	1,400	0.035	0.052
July 31	17.9	19.1	.013	.027	.16	.32	400	800	.014	.033
Aug. 28	20.4	19.1	.010	.031	.12	.37	300	1,000	.014	.038
Oct. 30	17.5	16.2	.033	.095	.40	1.15	1,000	3,000	.035	.087
Dec. 26	17.0	16.5	.039	.068	.47	.82	1,200	2,100	.039	.065
Feb. 19	16.1	15.0	.063	.123	.76	1.49	2,000	3,800	.057	.097
May 7	15.7	20.5	.043	.060	.52	.72	1,300	1,900	.037	.082

It is strikingly evident from these data (Table III) that the freezing-point depressions are not constant during the season, and that the concentrations of the soil solution are uniformly lower in the cropped soils than in the same soils uncropped. This latter observation is especially noteworthy, since comparisons of two samples of the same soil are peculiarly applicable. Any errors due to unfree water, type of soil, optimum moisture content, etc., would be practically constant. The same general relations hold, whether the original data are considered, or the corrected figures as used in the graphs. The only difference is that more exact comparisons may be made when the moisture contents are reduced to a uniform percentage.

The logical conclusion from the results given in Table III is that the concentration of the soil solution may vary widely at different periods of the year, and that the growth of the plant has a pronounced effect in lowering the concentration. Furthermore, this effect is of long duration. The soils which had been cropped are still decidedly lower in the concentration of the soil solution, as compared with the fallowed soils, eight months after harvesting the crop. It does not necessarily follow that the differences between the cropped and the uncropped soils are to be ascribed solely to the depletion of the soil solution by the plant. The evidence presented by Burd (*3*) is confirmatory of the idea that certain biological activities are more intense in the soil without crop, as shown by the increased production of nitrates and greater solubility of calcium and magnesium. It is nevertheless true that the lowest concentrations in the cropped soils occur at about the time when the crop has completed its maximum absorption of nutrients. In certain of the fine sandy loams the concentrations have been reduced to a very low point, corresponding to only a few hundred parts per million of total solids. This condition must be the result of withdrawals by the plant. The magnitudes of absorption, as given by Burd, are quite comparable with quantities present in the soil solution. After the minimum has been reached at the height of the growing season, the freezing-point depressions slowly increase up to the last date recorded in the graphs, May 1. It is highly probable that several weeks after cultivation a more marked increase in the concentration of the soil solution would take place.

At this point it is desirable to compare the data presented by Stewart (*8*) for the water extracts with the determinations made by the freezing-point method. It will be noted that the same general relations obtain in both cases. All the elements for which analysis was made are subject to marked fluctuations, with the exception of phosphorus. This subject will be given further consideration later in the article.

VARIOUS FACTORS AFFECTING THE SOIL SOLUTIONS

As a result of the observations on the tank soils which have just been described, it was decided to study various other factors possibly affecting the depression of the freezing point. What conditions other than cropping might increase or decrease concentration? The first step was to study the soils which had been held in a nearly air-dry state in bins. The results of these determinations have already been set forth in Table II, and comparisons may now be made between tank soils and the corresponding bin soils. It will at once be noted that a number of the latter show very much higher concentrations of the soil solution than either the cropped or the uncropped soils during most of the season. All three portions of each soil were originally derived from the same sifted and homogenous mass of soil. The existing differences must therefore be due to subsequent treatments which are correlated with three levels of concentration: (1) A very low concentration in the cropped soil, (2) a higher one in the uncropped soil, and (3) highest of all in some of the stored soils. The latter in a number of cases gave four or five times as great a depression of the freezing point as the corresponding cropped soils. The fact that the uncropped soils in the tanks had in some cases a less concentration of the soil solution than the stored soils may be explained by the treatment of the preceding year when both tank soils were cropped. It may also be true that long storage of the bin soils under the special conditions has brought about a decomposition of soil minerals as well as an increase in nitrates, with the result that a more highly concentrated soil solution is produced as soon as the soil is mixed with its optimum quantity of water. The extractions of the bin soils confirmed the freezing-point results. Water extracts showed a correspondingly high solubility for nearly all constituents.

With the establishment of the relations of freezing-point lowerings of soils under several conditions it became of interest to make a somewhat more detailed study in an effort to determine what factors are especially active in changing the concentration of the soil solution.

EFFECT OF INCUBATION ON CONCENTRATION OF SOIL SOLUTION

The first experiments dealt with the effect of long standing at laboratory temperatures. Samples were obtained from the tank soils, and after the determinations of freezing-point lowerings portions were kept in tight Mason jars for from one to three months. At the end of the specified periods the freezing points were again determined, with the results given in Table IV.

TABLE IV.—*Effect of incubation at laboratory temperature on depressions of freezing point*

Soil No.	Moisture.	Freezing-point depression.		Soil No.	Moisture.	Freezing-point depression.		Soil No.	Moisture.	Freezing-point depression.	
		Dec. 27	Mar. 29			Dec. 19	Jan. 9–16			Oct. 23	Nov. 3
	Per cent	*°C.*	*°C.*		*Per cent*	*°C.*	*°C.*		*Per cent*	*°C.*	*°C.*
4A....	19.3	0.055	0.073	1A...	23.4	0.037	0.050	10B..	13.6	0.099	0.439
4B....	18.0	.121	.133	1B...	22.3	.095	.152				
5A....	18.2	.066	.127	2A...	20.4	.047	.050				
5B....	18.0	.106	.153	2B...	20.0	.087	.100				
										Dec. 5	Dec. 13
6A....	24.5	.085	.120	3A...	20.9	.048	.063				
6B....	24.8	.103	.149	3B...	20.7	.060	.110				
7A....	15.9	.038	.052	8A...	12.7	.049	.062	10B..	12.8	0.153	0.180
7B....	14.7	.108	.081	8B...	12.5	.111	.142	10B..	14.4	.127	.169
12A...	13.9	.030	.013	9A...	11.0	.031	.038	10B..	14.6	.120	.148
12B...	11.7	.059	.046	9B...	10.6	.058	.072	10B..	16.0	.092	.114
14A...	17.0	.039	.091	10A..	15.1	.046	.058	10B..	17.0	.080	.104
14B...	16.5	.068	.096	10B..	14.6	.079	.105	10B..	17.8	.074	.097

It will be evident from Table IV that in the majority of cases there has been a distinct increase in the depression of the freezing point under the conditions of storage. It is significant that at the end of the storage period the uncropped soils still maintain their superiority with regard to the concentration of the soil solution. The time at which the sample is taken may in some cases influence the further changes taking place at laboratory temperatures. This suggestion is supported by the data for soil 10B. The samples taken in November gave a much greater increase in concentration after standing than was the case with the sample taken in December. Doubtless these differences may be associated with conditions favorable for nitrate production, but the extraction experiments have shown that other elements are increased in solubility simultaneously with the increased production of nitrates.

EFFECT OF CARBON-DIOXID GAS ON CONCENTRATION OF SOIL SOLUTION

The increase in concentration of the soil solution noted above, as well as the seasonal changes, may probably be related to the activities of microorganisms and their production of carbon dioxid. Several experiments were therefore undertaken to demonstrate the effect of carbon dioxid gas on freezing-point depressions. A sample of soil 5A containing 17.7 per cent of water was placed in a stoppered bottle, and a current of carbon-dioxid gas was passed through the moist soil for about five minutes. This treatment was repeated several times. The bottle was then closed tightly and the soil permitted to stand overnight in contact with the atmosphere of carbon dioxid, after which the depression of the freezing point was determined and compared with another sample of the soil exactly the same but not treated with carbon dioxid. The control sample gave a depression of 0.083° C. and the treated soil 0.138°.

A similar experiment was performed on the 5B soil, containing 16.7 per cent of moisture. The results in this case were 0.156° depression for the control and 0.218° for the sample treated with carbon dioxid. It is evident from these data that the gas has a striking effect in increasing the concentration of the soil solution. Here, again, it is interesting to observe that the cropped and uncropped soils maintain the same general relation to each other, even after the treatment described. Water extractions made on the same soils showed that the total water-soluble material was considerably increased by the carbon-dioxid treatment. Calcium was particularly affected, but several other elements were also made more soluble.

EFFECT OF DRYING ON CONCENTRATION OF SOIL SOLUTION

One of the important influences which may affect the chemical state of the soil is that of drying. King (*4*) has shown that drying soils in the oven causes a considerable increase in the quantity of water-soluble material. Bouyoucos and McCool (*2*) have pointed out that the evidence obtained by their freezing-point method tends to the same conclusion. The writers have desired in the present research to apply this method to the study of the influence of air-drying on the soils from the tanks. Freezing-point depressions were first determined upon the moist soils, and samples were then spread out in the laboratory and allowed to become thoroughly air-dried. They were then mixed in a mortar with suitable quantities of distilled water, allowed to remain in closed jars for several hours, and the freezing points, as well as the percentages of moisture again determined. The results are given in Table V.

TABLE V.—*Effect of drying on freezing-point depressions*

Soil No.	Moisture.	Freezing-point depressions.	
		Original soil.	After drying and remoistening.[a]
	Per cent.	°*C.*	°*C.*
1A	22.7	0.056	0.050
1B	20.1	.108	.191
4A	17.6	.098	.089
4B	17.2	.148	.114
5A	17.7	.083	.068
5B	16.7	.156	.100
8A	10.6	.062	.058
8B	10.8	.135	.142
9A	9.4	.057	.083
9B	9.5	.087	.126
10A	14.1	.066	.094
10B	13.4	.125	.136
11A	13.3	.065	.067
11B	13.5	.133	.110

[a] Calculated to same moisture content as original sample.

It will be noted that there are no marked or consistent changes in the freezing-point depressions as a result of the air-drying. In samples 5A and 5B water extractions (1 to 5) were made with the result that only negligible increases occurred in the quantity of any of the constituents, with the exception of the organic matter which was made somewhat more soluble. It may be suggested that a further increase in the concentration of the soil solution might subsequently be caused through stimulation of bacterial activity caused by the additional organic nutriment made available. In this case the solubility of various elements would be increased indirectly by drying, but there is no evidence that nutrient elements become more soluble by the simple process of drying at ordinary temperatures.

EXPERIMENTS WITH LEACHED SOILS

All of the foregoing work has consistently indicated that a given soil may yield very different soil solutions under different conditions. In order to establish the lower limits, it was decided to leach several soils with distilled water and then determine the depression of the freezing point. These were determined immediately after leaching and at intervals over several months, during which time the soils were kept at laboratory temperature in closed jars. Table VI shows the results obtained.

TABLE VI.—*Effect of leaching on freezing-point depressions*

Soil No.	Treatment.	Freezing-point depression. Before leaching. Determined.	Before leaching. Water.	After leaching. Date.	After leaching. Determined.	After leaching. Water.
		°*C.*	*Per cent.*		°*C.*	*Per cent.*
2B	800 gm. leached with 2 liters of water.	0. 117	21. 0	Nov. 3	0. 034	20. 4
				Nov. 8	. 021	
				Dec. 5	. 033	
				Feb. 26	. 034	
				May 2	. 030	
10B	do.	. 306	17. 2	Nov. 3	. 027	17. 2
				Nov. 8	. 020	
				Dec. 5	. 032	
				Feb. 26	. 040	
				May 2	. 051	
8C	1,000 gm. leached with 10 liters of water.	. 207	14. 3	Jan. 9	. 004	17. 6
				Feb. 6	. 006	
				Feb. 26	. 012	
				May 2	. 043	
5C	do.	. 060	21. 5	Feb. 6	. 013	22. 4
				Feb. 26	. 049	
				May 2	. 064	

It seems quite clear from an inspection of these data that the concentration of the soil solution may be reduced to an extremely low point through leaching and that this condition is maintained over a considerable period of time. Leaching has been shown by Lipman (5) to inhibit bacterial action markedly, and in the absence of such activity, it seems that the ability of the soil to recover its high level of concentration is very limited.

RELATION OF WATER EXTRACTS TO SOIL SOLUTION

Before entering upon the final discussion it is desirable to consider at this point certain more detailed relations of water extracts to the soil solutions, as indicated by the freezing-point method. The work of Bouyoucos and McCool enables us to obtain very useful and interesting estimates of the concentration of the soil solution, but obviously it is only the total concentration which is measured. We are quite unable to predict the nature of the substances whose resultant effect is expressed by the lowering of the freezing point. Only by some method of water extraction is it possible to gain any insight into the proportions of individual elements. Various comparisons between osmotic pressures in the soil solution and concentrations in water extracts may, however, be made, which, in certain directions, make possible very interesting deductions.

When different soils are to be compared, the first inquiry must be concerned with the amount of lowering of the freezing point which occurs for each 100 p. p. m. of total solids. This phase of the question has already been investigated by Bouyoucos and McCool, who found that extracts of varied types of soil gave very nearly constant results, approximately 0.0032° C. depression for each 100 p. p. m. of total solids in the solution. The writers have obtained closely similar figures for the soils used in this investigation as is made evident by the following data:

Freezing-point depression per 100 p. p. m. of total solids

Soil No.	°C.
2	0.0032
4	.0031
9	.0032
10	.0030
8	.0034
5	.0030
Average	.0032

By the use of this constant the approximate strength of the soil solution in terms of parts per million of total solids may be calculated, and this has been done in Table VII.

TABLE VII.—*Comparison of total solids in soil solution as calculated by freezing-point and extraction methods*

Date.	Soil No.	Total moisture.	Free water.	Freezing-point depression.	Concentration of total dissolved solids calculated from freezing-point depression.	Concentration of total dissolved solids calculated from extracts to free water.	Total dissolved solids in 100 grams of moist soil calculated from freezing-point depression.	Total sissolved solids in 100 grams of moist soil calculated from extracts.
		Per ct.	*Per ct.*	°*C.*	*P. p. m.*	*P. p. m.*	*Gm.*	*Gm.*
Nov. 15	4A	17.3	4.3	0.068	2,100	10,000	0.009	0.043
Do	4B	16.7	3.7	.082	2,600	15,000	.010	.055
Do	5A	16.8	3.8	.079	2,500	7,500	.010	.029
Do	5B	17.5	4.5	.088	2,800	8,900	.013	.040
Do	6A	24.1	6.4	.068	2,100	4,900	.013	.031
Do	6B	24.4	6.7	.101	3,200	6,900	.021	.046
Do	7A	14.9	8.9	.082	2,600	3,400	.023	.030
Do	7B	13.6	7.6	.151	4,700	6,400	.036	.049
Do	12A	14.4	10.4	.061	1,900	1,500	.020	.016
Do	12B	11.4	7.4	.138	4,300	4,800	.032	.036
Do	14A	17.5	10.0	.033	1,000	2,800	.010	.028
Do	14B	16.2	8.7	.095	3,000	6,300	.026	.055
Dec. 21	1A	23.4	5.4	.037	1,200	4,800	.006	.026
Do	1B	22.3	4.3	.095	3,000	12,400	.013	.053
Do	2A	20.4	7.4	.047	1,500	2,800	.011	.021
Do	2B	20.0	7.0	.087	2,700	6,300	.019	.044
Do	3A	20.9	6.9	.048	1,500	4,200	.010	.029
Do	3B	20.7	6.7	.060	1,900	5,600	.013	.038
Do	8A	12.7	8.7	.049	1,500	3,200	.013	.028
Do	8B	12.5	8.5	.111	3,500	7,500	.030	.064
Do	9A	11.0	5.0	.031	1,000	3,700	.005	.019
Do	9B	10.6	4.6	.058	1,800	6,200	.008	.029
Do	10A	15.1	7.1	.046	1,400	2,800	.010	.020
Do	10B	14.6	6.6	.079	2,500	5,700	.017	.038
Do	11A	14.5	8.5	.047	1,500	3,600	.013	.031
Do	11B	13.6	7.6	.134	4,200	6,600	.032	.050
Dec. 27	4A	19.3	6.3	.055	1,700	8,300	.011	.052
Do	4B	18.0	5.0	.121	3,800	14,800	.019	.074
Do	5A	18.2	5.2	.066	2,100	7,700	.011	.040
Do	5B	18.0	5.0	.106	3,300	13,300	.017	.067
Do	6A	24.5	6.8	.085	2,700	6,100	.018	.042
Do	6B	24.8	7.1	.103	3,200	10,100	.023	.072
Do	7A	15.9	9.9	.038	1,200	2,700	.012	.027
Do	7B	14.7	8.7	.108	3,400	7,800	.030	.068
Do	12A	13.9	9.9	.030	900	2,400	.009	.024
Do	12B	12.7	7.7	.059	1,800	4,300	.014	.033
Do	14A	17.0	9.5	.039	1,200	3,400	.011	.032
Do	14B	16.5	9.0	.068	2,100	4,800	.019	.043
Feb. 12	1A	22.7	4.2	.056	1,700	10,800	.007	.045
Do	1B	21.7	3.2	.108	3,400	21,700	.011	.069
Do	2A	20.2	7.2	.056	1,700	6,700	.012	.049
Do	2B	20.2	7.2	.101	3,200	7,600	.023	.055
Do	3A	20.6	6.6	.062	1,900	5,300	.013	.035
Do	3B	19.9	6.9	.120	3,800	6,100	.026	.042
Do	8A	10.6	6.6	.062	1,900	4,800	.013	.032
Do	8B	10.8	6.8	.135	4,200	7,900	.029	.054
Do	9A	9.4	3.4	.057	1,800	8,600	.006	.029
Do	9B	9.5	3.5	.087	2,700	11,100	.009	.039
Do	10A	14.1	6.1	.066	2,100	4,900	.013	.030
Do	10B	13.4	5.4	.125	3,900	9,400	.021	.051
Do	11A	13.3	7.3	.065	2,000	4,900	.015	.036
Do	11B	13.5	7.5	.133	4,200	8,300	.032	.062

TABLE VII.—*Comparison of total solids in soil solution as calculated by freezing-point and extraction methods*—Continued

Date.	Soil No.	Total moisture.	Free water.	Freezing-point depression.	Concentrated total dissolved solids calculated from freezing-point depression.	Concentrated total dissolved solids calculated from extracts to free water.	Total dissolved solids in 100 grams of moist soil calculated from freezing-point depression.	Total dissolved solids in 100 grams of moist soil calculated from extracts.
		Per ct.	*Per ct.*	°C.	*P. p. m.*	*P. p. m.*	*Gm.*	*Gm.*
Feb. 19	4A	17.6	4.6	0.098	3,100	8,800	0.014	.041
Do	4B	17.2	4.2	.148	4,600	16,000	.019	.067
Do	5A	18.1	5.1	.106	3,300	7,100	.017	.036
Do	5B	17.2	4.2	.115	3,600	11,900	.015	.050
Do	6A	24.8	7.1	.066	2,100	4,900	.015	.035
Do	6B	25.0	7.3	.126	3,900	7,500	.029	.055
Do	7A	14.6	8.6	.053	1,700	2,900	.015	.025
Do	7B	14.0	8.0	.093	2,900	5,300	.023	.042
Do	12A	12.7	8.7	.043	1,300	2,300	.011	.020
Do	12B	11.4	7.4	.083	2,600	3,900	.019	.029
Do	14A	16.1	8.6	.063	2,000	3,900	.017	.034
Do	14B	15.0	7.5	.123	3,900	7,200	.029	.054

Upon several occasions the total solids were determined in the 1-to-5 extracts of all soils. These were also calculated in terms of concentration of total soil moisture, as well as of free soil water. From the depressions of the freezing point, calculations were made of concentrations expressed as total solids in parts per million of the soil solution with the constant just described (Table VII). The first and most striking observation based on these results is that the relation between the total solids in the extracts of cropped and uncropped soils is also manifested by the depressions of the freezing point. In fact, throughout the experiment this correlation was noted. The fluctuations of the important nutrient elements in the water extracts already described by Stewart were in general agreement with the changes in the concentration of the soil solution. The low and high points came at about the same periods of the growing season in both cases.

From these considerations it might seem justifiable to assume that the material present in the actual soil solution forms at least an important fraction of the total solids dissolved in the 1-to-5 extract. It is, however, highly improbable that an extract should consist exclusively of the material present in the soil solution. Necessarily an additional quota would be derived from other more or less soluble substances, and the quantity dissolved would depend upon the conditions of extraction. A number of years ago Mitscherlich (*6*), in an important study on extracts of soils prepared with carbonated water, emphasized this point of view. His procedure consisted in making extracts with varying proportions of carbonated water, ranging from 5 to 1 up to 30 to 1. From the data so

obtained he constructed graphs and proved that with increasing quantities of water more material was dissolved. He therefore divided the total dissolved solids into two fractions: (a) That part soluble even with low moisture content, and (b) the additional material dissolved by the excess of solvent.

A number of difficulties arise in connection with this method of arriving at the concentration of soil solution. In the first place, it is not safe to assume that a curve based on one range of extractions can accurately be extended to cover another range of extractions. Indeed, the experimental data indicate that with the smaller proportions of water the curves may change their direction very appreciably and it is unfortunately impracticable to obtain extracts for analysis in those concentrations which correspond to optimum moisture conditions. Another limiting factor, previously neglected, has been described by Stewart. This concerns the differential effect of the solvent. The actual solvent in any case is not pure water, but pure water plus the solids already dissolved in the soil solution, and these vary enormously with changing conditions, even in the same soil. It is quite obvious that this factor would modify any calculations of the concentration of the soil solution based on water extracts. Moreover, a question may arise with regard to the relation of the soil extract to the residual solution. It is possible that the equilibrium may not be the same for the low and high proportions of water, especially in soils of colloidal character.

Notwithstanding these difficulties met with in attempting to predict accurately the concentration of the soil solution from soil extracts, it is still possible to make certain valuable comparisons of the two methods. If we should contrast the concentration of the soil solution, calculated from the extracts to the total moisture of the soil, with the concentrations shown by the freezing-point method, there would be a general similarity of magnitude. Logically, however, a comparison is much more justifiable when the extracts are calculated not to the total soil water, but to the free water, in the sense meant by Bouyoucos. It is then apparent that the concentration of the soil solution calculated by the extraction method is from two to five times that indicated by the depressions of the freezing point (Table VII). In other words, there is a considerably greater quantity of total solids dissolved in the 1-to-5 extract than is actually present in the soil solution at any given moment. The general order of magnitude of the two quantities is, however, evidently not disproportionate, the material extracted from the sandy loams averaging only about twice the quantity actually present in the soil solution, as shown by the freezing-point method.

The total amounts of dissolved material in 100 gm. of soil have been calculated by multiplying the percentages of free water by the concentrations in terms of parts per million of total solids. If we use the

freezing-point depressions as a basis, it appears that from 0.01 to 0.03 gm. of total solids is in solution for each 100 gm. of moist soil, while the total solids obtained by 1-to-5 extractions vary from 0.02 to 0.06 gm. per 100 gm. of soil.

In order to obtain approximate estimates of the material dissolved in an extract additional to that present in the soil solution, two soils (No. 5 and 8) were subjected to the procedure of successive extraction. One kgm. of each soil was extracted with a total of 10 liters of distilled water. Ten extractions were made, with 1 liter of water for each extraction. The results of the 10 extracts are plotted in figure 9. It will be noted that the first extract contained a very much greater quantity of dissolved solids than any subsequent extract. It was suggested by Stewart that some idea of the extra dissolved material might be formed by using as a basis of calculation the quantity dissolved in each liter after the extracts had become relatively constant. Thus, for soil 5 roughly 150 p. p. m. of total solids were found in each of the later extracts, and for soil 8, 100 p. p. m. By assuming that the rate of solubility is uniform, in a 1-to-5 extract there would be 5 times 150, or 750, p. p. m. of extra material dissolved in the case of soil 5, and 500 p. p. m. for soil 8. The total solids dissolved from 1 kgm. of soil by 5 liters of water aggregate 1,300 and 1,100 p. p. m. of total solids, respectively. This means that not more than 50 per cent could have originally been present in the soil solution. If this correction were applied to the extracts, the agreement with the data obtained by the freezing-point method would in some cases be fairly close, but usually the extracts would still give somewhat higher results. This fact is reasonably explained on the assumption that the first liter of solvent has dissolved out some material relatively soluble although not actually present in the soil solution, while the later extracts contain only difficultly soluble substances.

GENERAL DISCUSSION

It will now be well to correlate certain broad relations which may be deduced from the data presented in this paper through some further reference to the principal investigation of this series by Stewart. It should be emphasized again that neither from the water extraction nor freezing-point methods is there any evidence that the soil solution has a constant composition. On the contrary, the soil solution even of the same soil may vary greatly under different conditions. The water-extraction method indicates that this variation may occur with all the principal nutrient elements with the exception of phosphorus. These data accord with the view expressed by Bouyoucos and McCool, that the soil solution should not be considered as saturated. The opposite conception as outlined in the earlier work of Whitney and Cameron (*9*) is not upheld by the present investigation. If the great excess of nearly insoluble minerals in a soil were the determining factor in the soil solu-

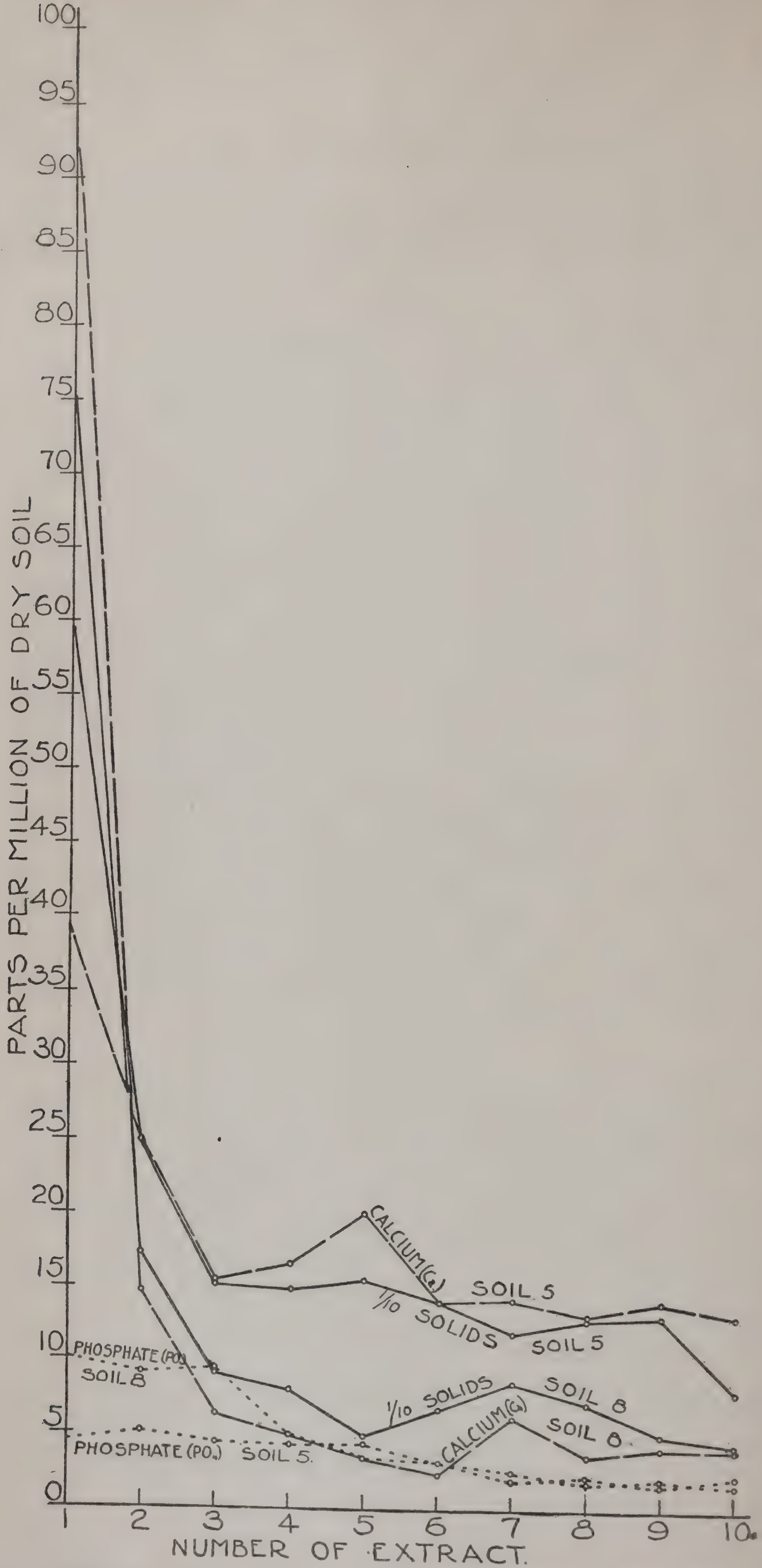

FIG. 9.—Graphs showing the results of successive extractions of soils 5 and 8. One liter of water to 1 kgm. of soil for each extraction was used.

tion, then its concentration in the cropped soil should not be strikingly diminished over a long period of time after the crop is removed, which in fact is the case. Also the soil solution in a leached soil has a greatly diminished concentration which is not markedly increased by long standing. In brief, the evidence is quite opposed to the theory that there is an immediate restoration of equilibrium when the soil solution is diminished in concentration by the plant or other agency.

One explanation of the fluctuations in the soil solution lies most probably in the varying nature of the solvent, especially in its content of carbon dioxid, which may well be one of the preponderating factors. The effect of carbon dioxid on the solution of soil minerals has been demonstrated by Mitscherlich, and the present investigation has indicated the effect of carbon dioxid in increasing the depression of the freezing point. It is generally conceded that the partial pressure of carbon dioxid in the soil atmosphere is greater than that of the outside air. Russell (7) has shown that the content of carbon dioxid in the soil may vary greatly at different times of the year, according to the intensity of bacterial action. In addition to carbon dioxid, other products of bacterial activity may have a further influence, the extent of which is still problematical.

It has been a general teaching of agricultural art that soil fertility is increased by those operations which tend to bring about optimum biological conditions in the soil. The experiments recorded in this investigation seem to afford direct evidence that the soil solution may be greatly affected by the activity of microorganisms and indirectly therefore by cultivation, temperature, organic matter, etc. Finally, the results of the experiments reported in this paper have considerable significance to the plant physiologist, since comparisons may be made between osmotic pressures in nutrient solutions and in the soil solution as it actually exists in the soil under conditions favorable to crop growth. It will be observed that in none of the soils did the plants obtain their nutriment from a highly concentrated solution. The general range of concentrations was from a maximum of 0.5 or 1.0 atmosphere to a minimum of 0.1 or 0.2 atmosphere. Under the conditions of approximately optimum moisture contents there was no sharp distinction between the silty clay loams and the fine sandy loams. By lowering the moisture content of the clay loams considerably, it is true that a very high concentration of the soil solution results, but under moisture conditions favorable to plant growth the solution is dilute. From certain of the soils stored in the bins a different idea of the concentration of the soil solution might be obtained, since this treatment has in some cases greatly increased the soluble material. The true conception of the nature of the nutrient media in soils can only be attained when soils are studied throughout the season under conditions suitable for crop growth.

Preliminary experiments have been carried out with the use of sand and water-culture methods with the nutrient solutions whose concentrations are comparable with those of the soil solution. The results have indicated that the osmotic pressures existing in the soil are also most favorable to the growth of barley in culture solutions. When considerably greater concentrations are maintained, decreased yields result, while very much lower osmotic pressures are suboptimum. Further experiments are now in progress.

It has not been possible in this investigation to make any general correlation with crop yield, although it is worthy of note that the two soils of lowest production, No. 9 and 12, show consistently low concentrations of the soil solution and also yield water extracts containing exceptionally small quantities of nutrient elements. In the discussions of Stewart and Burd the relations of chemical analysis of the medium to crop production are discussed more completely.

SUMMARY

(1) Freezing-point depressions have been determined on 13 soils under a variety of conditions.

(2) The concentration of the soil solution has been found to vary with the season and also as a result of treatment with carbon dioxid, leaching, incubation, etc.

(3) The growth of a crop markedly diminishes the concentration of the soil solution. This effect is still evident at the beginning of the following season.

(4) The soil solutions under conditions favorable to crop growth were found to be very dilute, particularly at the height of the growing season.

(5) Certain general agreements between the extraction and freezing-point methods are discussed.

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EFFICACY OF SOME ANTHELMINTICS

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INTRODUCTION

Although the use of anthelmintic treatment is an old practice in human and veterinary medicine, the efficacy of the various substances employed as anthelmintics is not well known. What information is available is based largely on clinical observations, efficacy being judged to a large extent on a consideration of the improvement or lack of improvement in the patient's health after treatment. In other instances the efficacy of the treatment has been checked by fecal examinations for worms passed and for eggs persisting in the feces; but, while this method gives real and valuable information, it is somewhat inexact. The methods employed for examining feces for worms passed are often rather casual; and negative findings in examining feces for eggs, especially when a small number of preparations are examined for only a few days after treatment, are not always conclusive.

A more satisfactory method of investigating anthelmintic efficacy is to administer treatment to animals, to collect all feces passed for a number of days, to recover from them all worms present, and then to kill the animals and collect all worms remaining. In this way it is possible to arrive at a fair idea of the anthelmintic effect to be expected from a drug, the correctness of the conclusions depending, of course, on the number of experimental animals used and their degree of infestation.

While it is thus possible to express the efficacy of a drug in the form of a mathematical ratio, the writers are fully aware that such ratios, except when based on extensive data, can not be considered an accurate index of the efficacy of the drug, since many factors, not entirely within control, such as the individual reaction of the animal, the amount of material in the alimentary tract, and the potency of the drug, all enter into the problem.

In carrying out this series of experiments the plan of the writers was to test as many drugs as possible having a known or alleged anthelmintic value, abandoning those which gave no results, and making further

[1] Resigned September 19, 1916.

experiments with the more promising. It is therefore possible that some of the drugs tested only once and on a limited number of animals may have more anthelmintic value than the tests indicate. On account of the extent of the field to be covered, the writers did not feel justified in devoting more effort to those drugs which gave small promise of success.

Some such method as the above has been employed by previous investigators. Hutcheon (*1891*)[1] made numerous tests of anthelmintic treatments for stomach worms in sheep and goats in South Africa, and followed the treatments by post-mortem examinations to determine the immediate effect on the worms. Stiles (*1901*, *1902*) did similar work in this country, and a number of veterinarians and stockmen made investigations involving treatment, post-mortem examination, and clinical observation. But, so far as the writers are aware at present, a detailed series of experiments covering the treatment of animals and the collection of all worms from the feces for a number of days up to the time of making a post-mortem examination in which all worms remaining were collected, has not been reported.

In this work the following method was pursued: The animals were given an appropriate dose of the anthelmintic to be tested, the method of dosage varying with the purpose of the experiment and the substance to be tested, preliminary purgation being undertaken or omitted as desired. Treatment was usually administered in the morning, and all feces were collected every morning thereafter until the animal was killed. The feces were washed through a set of graded screens and the screens examined for worms. The animal was usually killed the morning of the fourth day after the administration of the last dose of the anthelmintic, and all parasites remaining were collected and counted.[2] The percentage of efficacy was then estimated from the number of worms found on post-mortem examination and that number plus the number passed after treatment. When preliminary purgation was resorted to, the feces were collected on the following day, the day of administering the anthelmintic, to ascertain what worms if any were removed by simple purgation.

For convenience we have arranged our experimental data in three groups: (1) Simple purgatives, (2) a group including anthelmintic shaving a mineral base and coal-tar products, and (3) a group covering the vegetable anthelmintics. Tables I to V of the discussion of results summarize the results of the experiments.

[1] Bibliographic citations in parentheses refer to "Literature cited," p. 446-447.

[2] In conducting these experiments the writers occasionally found on post-mortem examination dead worms in the large intestine or rectum which normally are found in the small intestine. In such cases, as the worms were evidently in the process of passing out, they are credited as being removed by the anthelmintic.

EXPERIMENTS WITH SIMPLE PURGATIVES

CALOMEL

For worms in dogs.—Calomel in fairly large single doses followed by castor oil proved inefficacious as a remedy for ascarids in dogs. Four infested pups weighing 3.6 to 4.5 kgm. were fasted from noon of the day before treatment, given 4 to 5 gm. of calomel, and on the day following treatment given 15 mils (milliliters) of castor oil. Three of the dogs passed no worms after treatment; one passed a single ascarid (*Belascaris marginata*). Two of the dogs which passed no worms when examined post-mortem showed 10 and 8 *B. marginata*, respectively. The treatment was so evidently inefficacious that the other two dogs were not sacrificed for post-mortem examination. The results of this experiment do not accord with the claims occasionally made as to the anthelmintic value of simple purgatives and cathartics.

CASTOR OIL

For worms in dogs.—Although the efficacy of castor oil as an anthelmintic was not tested as a separate experiment, in several experiments it was given as a preliminary purge. The feces passed following its use were examined, and the worms passed compared with the number found post-mortem. In these experiments only 24 hours were allowed between the administration of the oil and the subsequent anthelmintic. It is therefore possible that the oil alone might have shown greater efficacy if the feces were collected for a longer time. However, in our experience the greater number of worms are passed within 24 hours after the administration of an anthelmintic; and in the case of a drug like castor oil, which usually acts within a few hours after its administration, it is not likely that the conclusions of the writers are much in error.

In 11 experiments in which castor oil was used as a preliminary purge on 50 dogs it removed 27 *B. marginata* out of a total of 351. Its effect on hookworms (*Ancylostoma caninum*) and whipworms (*Trichuris depressiuscula*) was practically nil. It was ineffective more frequently than it was effective, and in no case did it remove all the ascarids from a single dog. It has therefore little to recommend it as an anthelmintic. It may, however, have some diagnostic value in veterinary practice to confirm a clinical diagnosis of ascarid infestation in dogs when a microscopic examination of the feces is impossible, though it is evident that even for this purpose it would not always be reliable.

When worms are passed as the result of the increased peristalsis due to a simple purgative, there is always the suspicion that the worms were dead and would have passed out anyway and that the purge merely hastened their removal.

EPSOM SALT

FOR WORMS IN DOGS.—Epsom salt was tested once as a preliminary purge on three dogs which were given the salt in molasses at the rate of 4 gm. to each 5 kilos of weight. It had no effect on ascarids (*Belascaris marginata*), hookworms (*Ancylostoma caninum*), whipworms (*Trichuris depressiuscula*), *Dipyidium caninum*, or *Taenia pisiformis*, all of which were found present at post-mortem examination. Epsom salt is extremely distasteful to dogs and is likely to promote vomiting. The salt is best given in a vehicle like molasses, which serves to disguise the taste. Aside from this objectionable feature, the salt is less effective in dogs than other purgatives, such as castor oil or calomel, and is not particularly useful in canine practice.

FOR WORMS IN HOGS.—Campbell (*1917*), in a discussion of the treatment of swine for worms, mentions the use by a western veterinarian of Epsom salt dissolved in the drinking water. He says that the hogs are kept away from water for 12 to 24 hours, in warm weather not so long, in cold weather even longer. One ounce (28.35 gm.) of the salt is allowed for pigs and up to eight ounces (226.79 gm.) for hogs, then twice this quantity is added to allow for waste. It is then given in the drinking trough. The pigs being thirsty drink it. This completes the treatment. A test of this treatment was made by the junior writer on two pigs weighing about 23 kgm. (50 pounds) each. The animals were kept in separate pens on board floors and deprived of both food and water for 24 hours. Each pig was then given 226.8 gm. (8 ounces) of Epsom salt dissolved in about 3.8 liters (1 gallon) of water and placed in the drinking troughs. The pigs took a few swallows of the water and refused to taste more, showing their resentment by overturning the troughs and spilling the contents. The experiment was therefore repeated a few days later, the troughs being nailed to the floor. After 24 hours without food or water the pigs were given the salts as before and as in the previous experiment, took only a few swallows, refusing to drink more. Although no other water was given them for the next 24 hours, the troughs remained apparently as full of the salt solution as at the beginning of the experiment. As the pigs had now been without food and had drunk practically no water for 48 hours, the salt water was removed and fresh water and food given. The examination of the feces during the next three days revealed no parasites excepting one nodular worm (*Oesophagostomum dentatum*). The pigs were not killed, as the experiment was evidently a failure. A microscopic examination of the last fecal samples collected showed numerous ascarid eggs.

As it was evident that the Epsom salt in a solution of the strength given was extremely distasteful, it was decided to repeat the experiment with a weaker solution which the pigs might be induced to drink if sufficiently thirsty. Accordingly, the pigs were again deprived of food and

water for 28 hours and then given a solution made by dissolving 0.45 kgm. (1 pound) of Epsom salt in 22.7 liters (6 gallons) of water. As the drinking troughs would hold only 3.8 liters (1 gallon) each, each trough when full contained 75.6 gm. of the salt in solution. It was intended to refill the troughs as fast as they were emptied until each pig had taken 11.35 liters (3 gallons) of the solution, or 226.8 gm. of Epsom salt.

As in the previous experiment, the pigs merely tasted the solution and refused to drink more. No other water was given them for the next 28 hours during which time they had two meals of dry feed. As the pigs had now been without a drink for 56 hours, the experiment was closed. Another microscopic examination of the feces passed at the close of the experiment revealed the presence of ascarids in both pigs.

It is evident from the above that a solution of Epsom salt, even when less than 2 per cent in strength, is so distasteful to pigs that they will not drink it even after a relatively long period of thirst. It may also be noted that 11.4 liters (3 gallons) of water is much more than a pig of 22.67 kgm. (50 pounds) weight could consume in a day even if very thirsty, so that even if the pigs had drunk the weaker solution the treatment would have had to be prolonged for two or three days, greatly decreasing the purgative effect to be expected from a single dose of a large quantity of Epsom salt.

In this connection it may be stated that in an experiment with oil of chenopodium on hogs, conducted some years previously by the junior writer, four hogs were given 113.4 gm. (4 ounces) of Epsom salt mixed with a bran-mash feed, all the animals eating together. No difficulty was experienced in getting the animals to eat the mixture, but the amount of the salt allowed to each hog was only one-eighth of the dose allowed in the present experiment.

EXPERIMENTS WITH ANTHELMINTICS OF A MINERAL NATURE AND COAL-TAR PRODUCTS

TARTAR EMETIC

FOR WORMS IN HOGS.—Tartar emetic is commonly used as a remedy for roundworms in horses, and this led the writers to test its effects upon worms in hogs. Two small pigs were used, weighing 8.6 and 14.5 kgm. They were each given 259 mgm. of tartar emetic dissolved in water, followed after a short interval with 29.57 mils of castor oil. Winslow (*1913*) gives the emetic dose of tartar emetic for hogs as 4 to 10 grains (259 to 648 mgm.). The drug in this case exerted no emetic effect. One of the pigs passed 5 ascarids (*Ascaris suum*) after treatment and showed the presence of 5 others on post-mortem; the other pig passed none and showed 19 on post-mortem. Owing to the more or less common habit which pigs have of devouring ascarids, it is possible that the second pig also passed worms which were eaten; and the first may have passed

more than were recovered in the feces saved after treatment. A few whipworms (*Trichuris suis*) and nodular worms (*Oesophagostomum dentatum*) were passed; a few of the former were found in one of the pigs post-mortem, not looked for in the other, and about 250 and 500 nodular worms were found, respectively, in the two pigs post-mortem.

This experiment demonstrated that tartar emetic has an anthelmintic action on worms in swine, but further trials will be necessary before conclusions can be drawn as to its efficacy. The method of administration used in the experiments is not likely to prove suitable in practice.

CHLOROFORM

Chloroform has been more or less used for some time, either alone or in combination with other substances, as an anthelmintic. Recently Alessandrini (*1915*) has commended it very highly for use against hookworms and other worms. He states that it has these advantages: Combined with castor oil it requires no special preparation of the patient; it can be administered in a single dose; it does not cause local or general disturbances either immediately or subsequently; it is perfectly well tolerated and is not nauseous. It may be given in 3- to 4-gm. doses dissolved in olive oil or castor oil and is thoroughly efficacious against hookworms, whipworms, pinworms, and ascarids. Chloroform is a constituent of Hermann's mixtures, the formula of one of which is given on page 403. Schultz (*1911*) finds the chloroform to be the active anthelmintic ingredient of Hermann's white mixture. He found chloroform very effective against hookworms in the dog and says of it:

> It has proven rapid in its action and thus far not followed by any evil after effects. Should the chloroform-castor oil mixture act as favorably in human beings as it has for me in dogs, it will prove a universal worm remedy of great importance.

In view of the irremediable damage resulting from overdoses of chloroform, Schultz is inclined, however, to favor the use of the less effective drug thymol. Billings and Hickey (*1916*) use a chloroform-castor-oil mixture following the use of chenopodium in the treatment of hookworm and other roundworm parasites of aliens detained at the immigrant station at Angel Island, California. For an adult they administer 20 mils of a mixture of chloroform and castor oil containing 1.8 mils of chloroform. The dose is graduated according to the apparent age.

The writers' experiments with chloroform as a drug for expelling hookworms from dogs inclined them to the belief that it was extremely satisfactory compared with thymol, and on the suggestion of Dr. Charles Wardell Stiles, of the United States Public Health Service, a note was sent to Dr. Reid Hunt, of Harvard University, asking his opinion as to the danger of the administration of chloroform. In his reply he states:

> It has been shown . . . . that even one administration of chloroform causes distinct changes in the liver, from which, however, animals almost always completely recover. I presume that a single dose is efficient and ordinarily harmless; but I think that if the

liver or heart had already been injured or diseased, a single does might have serious consequences. It would seem advisable not to repeat a dose for several days at least.

The experiments of the writers with chloroform were as follows:

FOR WORMS IN DOGS.—Five dogs, weighing from 5.5 to 17.7 kgm., were given, in the morning after fasting from the evening of the day before, 0.2 mil of chloroform per kilo of body weight mixed with 3 mils of castor oil per kilo of body weight. All of the dogs were infested with hookworms (*Ancylostoma caninum*), four very lightly, one heavily, three had a few ascarids (*Belascaris marginata*), four a few (1 to 27) whipworms (*Trichuris depressiuscula*), two a few tapeworms (*Dipylidium caninum*), and one numerous (250) tapeworms of the same species. The dog which had the single hookworm failed to lose the parasite as a result of the treatment, but none were left in the other lightly infested dogs, while more than half the hookworms were passed by the heavily infested dog (457 passed and 355 found post-mortem). One dog passed a single ascarid and showed none post-mortem, two others showed 1 and 2 ascarids, respectively, post-mortem, but had passed none after treatment. Two of the dogs out of the four infested with whipworms passed a few of these worms, and all four showed a few on post-mortem. After the treatment no tapeworms were recovered from the feces of the dogs infested with *D. caninum*.

In this experiment chloroform and castor oil proved rather highly efficacious in removing hookworms, removing all in three cases of light infestation, failed to remove any in one case, and removed over half of the parasites in a case of heavy infestation. The results in the case of ascarids and whipworms are not striking. In the case of *D. caninum* there is no evidence that chloroform is of value as an anthelmintic.

The failure of chloroform to remove all the hookworms from the heavily infested dog indicates that repeated treatments may be necessary in cases of heavy infestations, but in view of the dangers attending the use of chloroform the advisability of repeating the dose is questionable. Several days at least, perhaps a week or longer, should be allowed to elapse to allow time for the animal to recover from the possible ill effects of the first treatment. Further experiments are desirable.

A further test of the efficacy of chloroform in combination with other drugs as an anthelmintic for hookworms in dogs was made with the remedy in the form of Hermann's mixture. This preparation consists, according to Railliet (*1915*), of the following:

Oleoresin of male-fern	4 gm., or
Oil of eucalyptus	2 gm.
Chloroform	3 gm.
Castor oil	40 gm.

He suggests its use as a substitute for thymol. Although the preparation is intended to include either male-fern (*Dryopteris filix-mas*) or eucalyptus oil, through an error both drugs were included. In conducting this experiment two objects were aimed at: (1) To determine whether

or not the chloroform was the sole or principal cause of the efficacy of the mixture as a remedy for hookworms (*Ancylostoma* spp.); (2) whether the combination of castor oil and male-fern was likely to produce symptoms of male-fern intoxication, owing to its greater degree of absorption when combined with an oil.

After a fast of 24 hours, two dogs weighing 9.5 and 16.5 kgm., respectively, were given the full dose of Herman's mixture as detailed above. Two other dogs weighing 9.6 and 12.4 kgm., respectively, were given the mixture without chloroform. Nearly three-fourths of the hookworms (*Ancylostoma caninum*) in the two dogs receiving the full mixture were eliminated by the treatment, one dog passing 16 *A. caninum* after the treatment and having only 3 left at the post-mortem examination. On the other hand, out of 39 *A. caninum* in the two dogs receiving the mixture without chloroform, only 3 were eliminated. The mixture without chloroform showed some slight efficacy for whipworms, but the figures are not striking. Two of the dogs were infested with *Dipylidium caninum*, one passing a number of fragments with several heads within 30 minutes after dosing. The other dog passed a few segments. Both dogs were found free from *D. caninum* at post-mortem examination. As these dogs had received the mixture without chloroform, it is evident that the tæniacidal value of the mixture was due to the male fern or eucalyptol and not to chloroform. This may reasonably be considered a demonstration of the efficacy of male fern as a tæniacide.

To sum up, it appears from the above that Hermann's mixture (including both eucalyptol and male-fern) is an efficient vermifuge for hook worms, owing largely, if not entirely, to the chloroform content. It is also efficacious for *Dipylidium caninum*, probably on account of the male fern it contains. It seemed to have some slight effect on whipworms, but the evidence of this was by no means convincing. The combination of male-fern and castor oil seemed to have no deleterious effect on the experiment animals, in this respect supporting the opinion of Lenhartz (*1902*) and Seifert (*1908*). Further experimentation, however, is desirable to elucidate this point.

For worms in sheep.—Chloroform was tested on two sheep weighing 25 and 28 kgm., respectively. The dose for each was mixed with 60 mils of castor oil, the smaller sheep receiving 5 mils of chloroform (0.2 mil per kilo) and the other 10 mils (nearly 0.4 mil per kilo). Both sheep died four days after dosing and showed, on post-mortem examination, lesions of gastro enteritis and pneumonia in the congestive stage. The sheep were very lightly infested with parasites. None were recovered from the feces of the sheep which received the smaller dose; three stomach worms (*Haemonchus contortus*), five nodular worms (*Oesophagostomum columbianum*), and five hookworms (*Bunostomum trigonocephalum*) were found in this sheep post-mortem. The sheep which received the larger dose passed nine stomach worms and one nodular worm after treatment, and two stomach worms were found post-mortem.

The chloroform had an evident anthelmintic effect on the sheep which received the larger dose. The fatal results of the treatment in both cases, however, indicate that chloroform is not a promising anthelmintic for use on sheep.

ETHER

FOR WORMS IN DOGS.—Four dogs weighing from 2.38 to 4.89 kgm. were given ether in a dose of 0.8 mil per kilo mixed with 15 mils of castor oil for the two smaller dogs and 30 mils of castor oil for the two larger dogs. The afternoon of the day before treatment the dogs were given a preliminary dose of castor oil, and were not fed until several hours after receiving the ether. No worms were recovered from the feces passed between the administration of the preliminary dose of castor oil and the administration of the ether and oil mixture. This mixture was much resented by the dogs, and all of them were salivated by it and showed more or less evidence of collapse. One of the dogs passed a few tapeworm segments (*Taenia pisiformis*), but no other tapeworm material was recovered from the feces; nor were any tapeworms found post-mortem. No hookworms were recovered from the feces, but all showed infestation on post-mortem examination, the number of worms being 7, 21, 233, and 242, respectively. One of the dogs passed 1 ascarid and one passed 6. At the post-mortem examination no ascarids were found in the former and 11 in the latter. The other two passed no ascarids; 4 were found post-mortem in one, but 1 of these was in the rectum, evidently about to be passed; 1 ascarid was found post-mortem in the other of these two dogs.

The conclusion from this experiment is that the ether exhibited a rather slight anthelmintic action against ascarids, no evident action against hookworms, and probably was instrumental in the removal of a tapeworm which was presumably present in one of the dogs, in view of the discovery of segments in the feces.

IODOFORM

FOR WORMS IN DOGS.—Among the remedies prescribed for ascarids in human subjects iodoform has been occasionally recommended. Schidlowski (*quoted by Seifert, 1885, p. 98*) gives it in the form of a powder mixed with sodium bicarbonate in doses of 0.01 to 0.06 gm. three times a day, followed by a dose of castor oil on the last day. In the present experiment it was intended to give the maximum amount, 0.18 gm., in one dose to each of four dogs ranging in weight from 11.8 to 14 kgm. However, through an error in weighing the drug, the dogs were each given 0.018 gm. instead of 0.18 gm. This error was discovered afterward, and the next day the full dose, 0.18 gm., was given. Thus, each dog received a total of about 0.2 gm. of iodoform, given in capsule with sodium bicarbonate. The dogs were starved for 24 hours before treatment, and allowed one meal between the first and second doses. After each dose 29.6 mils of castor oil were administered. One Taenia segment was passed by one

of the dogs, no worms being recovered. As the treatment was obviously inefficacious, only two of the dogs were killed. The post-mortem examination showed these animals infested with various numbers of intestinal nematodes and tapeworms. As far as can be judged from a single experiment, iodoform is valueless as an anthelmintic for intestinal parasites in dogs, even when given in doses in excess of that prescribed for human subjects.

COPPER SULPHATE

For worms in sheep.—The use of copper sulphate as an anthelmintic against stomach worms in lambs was developed by Hutcheon (*1892*, *1895*), who reported thousands of cases of its successful use in South Africa. His favorable reports were based largely on clinical findings, but in a number of cases he treated animals with the copper-sulphate solution and killed them a short time afterward to determine whether the worms in the stomach were dead or alive. The solution he used is approximately that which would be obtained from 0.45 kgm. of pure copper sulphate, powdered fine and dissolved in 35.96 liters of warm water. Only clear blue crystals are used, and it is best to powder these and then to dissolve the powder in a small quantity of hot water and to add cold water to make up the required amount. He gave the solution in the following doses.

Lambs 3 months old	¾ ounce (22. 17 mils).
Lambs 6 months old	1½ ounces (44. 56 mils).
Sheep 12 months old	2½ ounces (73. 9 mils).
Sheep 18 months old	3 ounces (88. 7 mils).
Sheep 24 months old	3½ ounces (103. 5 mils).

Stiles and others have tested and recommended copper sulphate in these doses.

It did not appear to the writers that such refinement in dosing was called for in treating sheep; therefore, for the sake of simplicity a 1 per cent solution was made up and administered in amounts of 100 mils to sheep a year old or older, and in amounts of 50 mils to lambs under a year old.

Five sheep less than a year old were dosed with copper sulphate, two receiving 0.5 gm. each of powdered copper sulphate in capsule, and three receiving 50 mils each of the 1 per cent solution.

The two sheep receiving powdered copper sulphate in a capsule passed a few stomach worms, and on post-mortem showed over 6,000 in one case and over 4,000 in the other. No nodular worms were recovered from the feces, but the post-mortem examination showed over 100 in one case and nearly 200 in the other. Both showed a few tapeworms (*Moniezia* sp.) and hookworms post-mortem, none having been recovered from the feces after treatment. Several other species of nematodes were also found in varying numbers at the post-mortem examination.

The three sheep which received the copper-sulphate solution passed, respectively, 120, 240, and 314 stomach worms, and showed on post-

mortem 0, 49, and 3, respectively. There was no marked effect upon the nodular worms, although one of the sheep passed 2 worms of this species, 175 were found post-mortem, and 143 and 21, respectively, were found in the two others, from whose feces none were recovered after treatment. Two of the sheep showed hookworms on post-mortem examination, and, although none were found in the other one, it is quite probable that none were present when the animal was treated, as none were recovered from the feces after treatment.

Evidently the powdered copper sulphate in capsule exhibited no anthelmintic action. On the other hand, supporting the experience of Hutcheon, Stiles, and others, a 1 per cent copper-sulphate solution in 50-mil doses proved very efficacious in the removal of stomach worms. It had no evident effect upon other intestinal parasites.

In view of the efficacy of the copper-sulphate solution, a test was made to determine how readily and easily it could be administered, with a view to determining whether large numbers of animals could readily be treated with the solution. A dosing apparatus devised by the senior writer was constructed and used as follows:

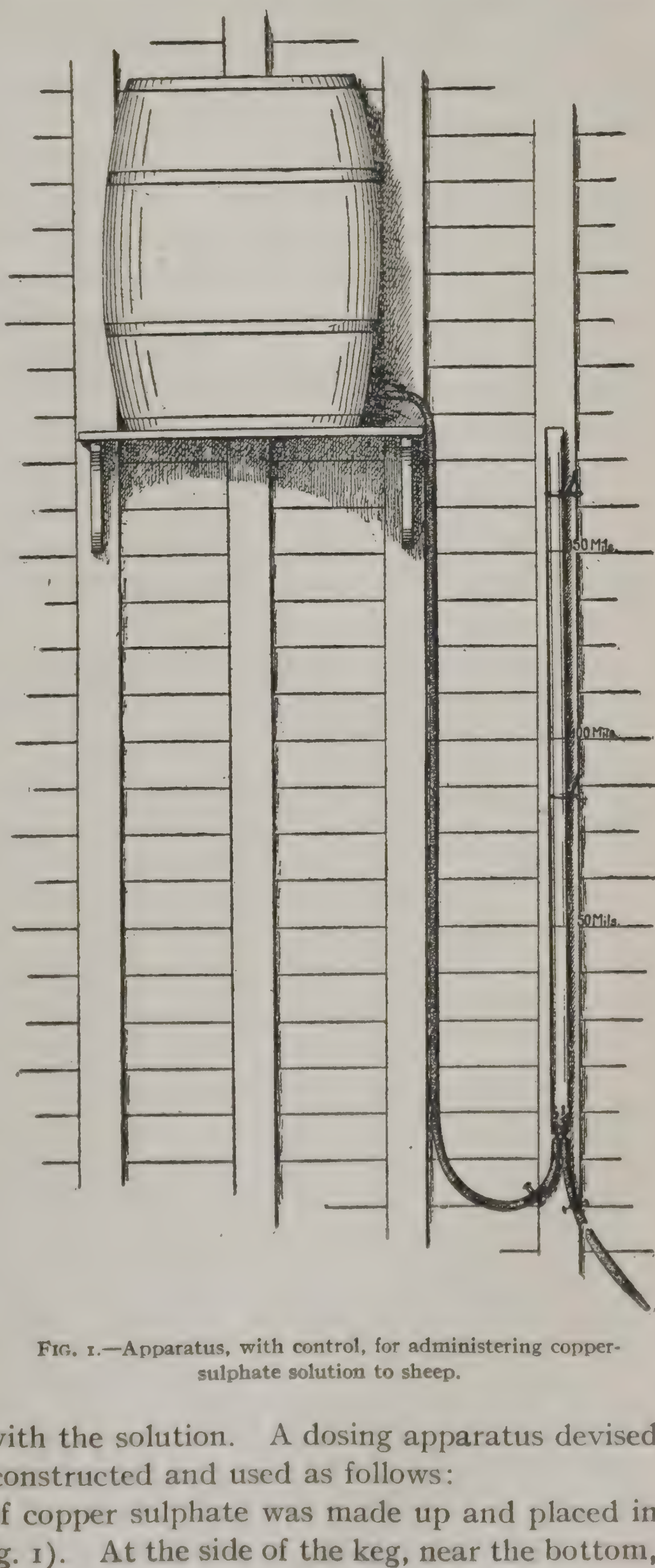

Fig. 1.—Apparatus, with control, for administering copper-sulphate solution to sheep.

A 1 per cent solution of copper sulphate was made up and placed in a small water-tight keg (fig. 1). At the side of the keg, near the bottom,

a perforated cork with a glass tube through the perforation, was inserted in an auger hole. A rubber tube was connected with the glass tube. The keg was placed on a high shelf in the barn and the rubber tube from the bottom of the keg connected with one of two glass tubes that perforated a rubber cork in the bottom of a graduated glass cylinder, the top of the cylinder being on a level with the bottom of the keg. The graduated glass cylinder was fastened by wires to two nails driven into the wall in such a way as to maintain it in a vertical position.

A second rubber tube was connected with the second glass tube in this cork, this tube terminating at the other end in a piece of metal tubing. The glass cylinder was graduated at 50-mil intervals and had a capacity of 150 mils. Close to this cylinder, pinch controls were fastened on the rubber tubing leading into and out of the cylinder. By pinching the control on the rubber tube connecting the keg with the glass cylinder, the copper-sulphate solution was allowed to flow from the keg to any desired graduation in the lower cylinder. By letting this close and pinching the other control, the solution was allowed to flow from the cylinder to the metal tubing at the other end of the dosing tube, the size of the dose delivered being noted on the cylinder. The metal tubing was held in the sheep's mouth by one man, while another man controlled the size of the dose.

In actual practice, with two men operating this apparatus, as noted, and with a third holding the sheep and a fourth bringing them up, 25 sheep were given 50-mil doses in 15 minutes, and 27 sheep were given 100-mil doses in 25 minutes, a total of 40 minutes for 52 sheep. In the 50-mil dose, which is perhaps enough for sheep of any age, since it combines safety with efficacy, sheep can be dosed at the rate of 1⅔ a minute. This is 100 an hour, or 800 sheep for an eight-hour day.

It should be noted that of the sheep that received 100-mil doses of the solution, the equivalent of 1 gm. of copper sulphate, 2 sheep died in the course of the next two days. These sheep were 10 months old, and these doses would appear to be too large. Neither of the dead sheep showed any indications of traumatic pneumonia, but the fourth stomach of one of them was much congested.

On a subsequent occasion the 50 sheep surviving from this experiment were dosed with 50-mil doses, using the apparatus described. No bad results of any sort were experienced. The sheep have been similarly treated subsequently, and occasional post-mortem examinations of the sheep in this lot show almost complete freedom from stomach worms.

COPPER SULPHATE

For worms in dogs.—In view of the efficacy of copper sulphate against stomach worms in sheep, an attempt was made to determine whether the well-known emetic action of this drug would entirely prevent its use as an anthelmintic for dogs. Four dogs were each given 0.5 gm. of copper

sulphate dissolved in 10, 20, 30, and 40 mils, respectively, of water, and 2½ hours later they were given 14.79 to 29.57 mils of castor oil each. There was prompt emesis in a few minutes after the administration of the copper sulphate. Fecal examinations for the following four days showed only 1 worm, a whipworm. Because of the evident defects of the treatment, the dogs were not killed and examined. It is obvious that the emetic action of copper sulphate precludes the use of this substance as an anthelmintic for dogs.

GASOLINE

FOR WORMS IN SHEEP.—Gasoline has been extensively used as a treatment for stomach worms, but some authorities consider that there are dangers attending its use. Stiles (*1901*) says of it:

> Gasoline has recently gained considerable reputation as a vermifuge. I have used it in a number of cases and have found the claims made for it to be more or less justified. Three objections, however, arise to its use, and I can not, therefore, consider it an ideal treatment. These objections are:
>
> (1) Not less than three doses, and usually four to six, are required to expel the worms.. Its use involves a great expenditure of labor, and it is, therefore, impracticable on the large ranches.
>
> (2) While several doses are not necessarily injurious to the stock, still, if the doses are large, repeated drenches cause a more or less severe congestion of the bowels. Not only that, but repeated handling of range sheep, with the necessary preliminary treatment of withholding food, is injurious to the animals.
>
> (3) If used on animals suffering from pleurisy, it is likely to be fatal. I have had several fatal cases of this kind.

Luckey (*1915*) says:

> Gasoline for acute cases is a specific. One dose is enough. * * * The average man can not give gasoline without killing the animal. One can not give a sheep with a little bit of pneumonia gasoline without killing it.

Arbuckle (*1916*) says of himself and Joe Wing:

> We were the first men in the country to employ the gasoline treatment successfully. Wing had heard of it as a remedy used in France. We were also among the first to discover that this was not a practicable treatment.

Gasoline is usually given in milk, linseed oil, or flaxseed tea, which makes the treatment considerably more expensive, and, as these feeds are not always available in large quantity, the treatment is not well adapted to the needs of large flocks.

Stiles (*1901*), Ransom (*1907*), and others have recommended ¼ ounce (7.39 mils) of gasoline as the dose for lambs and ½ ounce (14.79 mils) as the dose for sheep. Coffey (*1915*) recommends larger doses; lambs at weaning to get ¼ ounce, ½ ounce, and ¾ ounce, respectively, on each of three successive days; sheep to receive ½ ounce, ¾ ounce, and 1 ounce, respectively, on each of three successive days, these doses to be given thoroughly emulsified in 5 ounces (147.87 mils) of milk linseed oil, or flaxseed tea.

In the writers' first experiment four lambs were treated. Two of them weighing 21.09 and 21.77 kgm., respectively, were given 7.39 mils of gasoline in 148 mils of milk at each dose, the equivalent of the dose commonly recommended, and the others, weighing 26.3 and 22.2 kgm., respectively, were given 14.8 mils of gasoline in 148 mils of milk at each dose, the equivalent of Coffey's doses of ¼, ½, and ¾ ounce for lambs. The dose named was given to the lambs on each of three successive days.

The two lambs which received 7.39 mils of gasoline passed about one-fourth of the total number of stomach worms (*Haemonchus contortus*) present, no hookworms (*Bunostomum trigonocephalum*), of which only a very few were present, and several nodular worms (*Oesophagostomum columbianum*), of which a considerable number were found post-mortem. There was no evident effect on various other species of nematodes and tapeworms present in one or both of the lambs. The two lambs which received 14.8-mil doses of gasoline were more lightly infested; one which passed 58 *H. contortus* showed none on post-mortem; the other passed 4 and showed 2 post-mortem. There was very little effect on hookworms present in small numbers in one of the lambs.

All four of the lambs showed lesions of pneumonia which were suspected to be of traumatic origin.

In the second experiment three sheep were treated. The larger dose of gasoline, recommended by Coffey and found most efficacious by the writers, was employed and was given on each of three successive days by a stomach tube passed down the esophagus, but not far enough to direct the fluid into the rumen. In this way the writers expected to avoid the possibility of causing traumatic pneumonia. None of the sheep passed any stomach worms, and none were found post-mortem, so the experiment throws no light on this subject. The treatment showed a slight efficacy for hookworms (*Bunostomum trigonocephalum*). It failed to remove any nodular worms (*Oesophagostomum columbianum*) from one sheep, but removed all specimens of this species from another lightly infested sheep. It apparently removed all specimens of *Cooperia* sp. from the only animal infested with this parasite.

From this experiment it would appear that gasoline has some slight effect on intestinal worms in lightly-infested sheep, but the evidence is not sufficient to justify its use for this purpose without further experimentation. No lesions of pneumonia were observed in this experiment, although the stomachs of all three sheep showed lesions suggesting healing ulcers with traces of hemorrhage.

As the question of the efficacy of gasoline as a vermifuge for stomach worms in sheep and the likelihood of this treatment's causing lesions of pneumonia and gastritis did not seem to be settled, a further trial of gasoline was carried out by the junior writer. In this experiment two

sheep were used and treated by drenching with increasing doses of gasoline in milk as recommended by Coffey (*1915*). The dosage given was 15, 22, and 30 mils in 236.6 mils of milk on the first, second, and third days, respectively. The same dose was given to each animal.

The first sheep passed no stomach worms and had none on postmortem examination, so the efficacy of the treatment in this case is undetermined. A few hookworms only were passed. The second sheep, which was lightly infested with stomach worms, passed none, but passed a few specimens of hookworms and nodular worms, forming a small percentage of the total number present.

In this experiment, although the sheep received much larger doses than in the previous experiments and the medicine was given in a drench, there were no evidences of pneumonia or gastritis.

Considering the three experiments with gasoline as a whole, involving the use of nine experiment animals, the writers find that this treatment removed over one-fourth of the stomach worms present and had some slight efficacy for hookworms and nodular worms. This compares very unfavorably with the efficacy of copper sulphate for stomach worms when given in solution. The gasoline treatment has also the further disadvantages that it must be given three times, and in a vehicle such as linseed tea or milk, which is an additional expense. There is also the possibility of causing traumatic pneumonia, although the subsequent experiments with this drug indicate that gasoline is not necessarily more dangerous as a drench than is copper sulphate.

PETROLEUM BENZIN

FOR WORMS IN SHEEP.—As already stated, Stiles (*1901*) was favorably impressed with the use of gasoline as a vermifuge in spite of certain objections that he notes. It was suggested by Dr. B. H. Ransom, Chief of the Zoological Division, Bureau of Animal Industry, that our failure to get more satisfactory results might be due to the difference in the commercial gasoline of the present day and that used by Stiles. It was recalled that in 1901, when Stiles conducted his experiments, the automobile industry in this country was in its infancy, and there was little demand for gasoline. Consequently the petroleum distillers included only the most volatile hydrocarbons in gasoline, reserving the heavier fluids for kerosene, their principal product. At the present day, with conditions reversed, the distillation temperatures of gasoline have been greatly extended, with a consequent increase in the specific gravity and lessening of volatility.

In order to determine whether the lessened volatility of the present-day gasoline was related to its inefficacy as a vermifuge, a test was made by the junior writer of petroleum benzin, U. S. P. This product, distilled between 45° and 60° C., represents only the most volatile hydro-

carbons of petroleum and is probably more like the commercial gasoline used by Stiles (*1901*) than the present-day commercial product.[1]

Two full-grown sheep were used for the experiment. The animals were starved for 24 hours and then given 15 mils of petroleum benzin in 150 mils of milk, on three consecutive days. They were drenched through a tube held in the mouth. In no case were any symptons of intoxication from the fumes of the benzin observed during or after its administration, and the post-mortem showed no lesions which could be attributed to the action of the drug.

The chief points of interest in this experiment are:

(1) The apparent superiority of refined gasoline (petroleum benzin) over commercial gasoline as an anthelmintic for stomach worms (*Haemonchus contortus*) and hookworms (*Bunostomum trigonocephalum*). It proved 88 per cent efficacious for stomach worms while the latter was only 35 per cent efficacious, as shown by the summary of three experiments. Its efficacy against hookworms is still more marked. It was 73 per cent efficacious for this parasite, while gasoline proved only 5 per cent efficacious.

(2) Its entire inefficacy against nodular worms (*Oesophagostomum columbianum*). No worms of this species were removed by petroleum benzin, while the three experiments with gasoline showed an efficacy of 16 per cent for this parasite.

The post-mortem examination of the two sheep showed that they were both very lightly infested with stomach worms (*Haemonchus contortus*), most of which had been removed by the treatment. The remedy removed all hookworms (*Bunostomum trigonocephalum*) from one sheep and more than half the number present from the second sheep. It was entirely inefficacious against other intestinal nematodes and tapeworms (*Moniezia* spp.). Its failure to destroy any of the nodular worms (*Oesophagostomum columbianum*) in the large intestine may be explained on the grounds that it is perhaps more readily absorbed than commercial gasoline, and, hence, was largely absorbed before reaching the colon. It should be pointed out that in this experiment the animals were starved for 24 hours before treatment, while in the experiments with gasoline they were not starved. This may have had something to do with the results notwithstanding the slowness with which the rumen becomes empty when sheep are starved.

In summary it may be stated that petroleum benzin showed marked superiority over commercial gasoline as an anthelmintic both for stomach worms (*H. contortus*) and hookworms (*B. trigonocephalum*). While it did not quite equal the treatment with the copper-sulphate drench as a

[1] According to the issue of the Journal of the American Medical Association for October 14, 1916, the present commercial gasoline includes hydrocarbons distilling at a temperature as high as 175° C. (TYDEMAN, F. W. L. NAPHTHALENE IN GASOLINE FOR AUTOMOBILES. *In* Jour. Amer. Med. Assoc., v. 67, no. 16, p. 1175.)

remedy for stomach worms, it was far superior to anything else tried as a remedy for hookworms (*B. trigonocephalum*) and is worthy of further experimentation to test its efficacy on more heavily infested animals. For the treatment of stomach worms copper sulphate must still be considered superior, not only because it is somewhat more efficacious but on account of its lesser cost and the fact that only one dose of copper sulphate is required, whereas three doses of petroleum benzin are advisable.

PHENOLS

The phenols a re a group of organic compounds composed of hydroxy derivatives of the benzene series, the hydroxyl radical being linked directly to the nucleus. The refined phenols include phenol (C_6H_5OH), commonly called carbolic acid; cresol ($C_6H_4CH_3OH$), commonly called cresylic acid or kresol; and the higher phenols. The term "crude phenols" is used in general to designate those unrefined mixtures of the phenols proper with certain hydrocarbon oils and other impurities with which they become associated in the course of their preparation, whether from coal-tar, wood-tar, or blast-furnace gases. There are on the market numbers of trade preparations in the form of soaps, powders, ointments, or liquids which contain refined or crude phenols as essential constituents. A number of these liquid phenol preparations have been used and recommended as anthelmintics. Some of these liquids are insoluble in water, in which case they may or may not be capable of being emulsified, and others are soluble in water.

For worms in sheep.—Three preparations were tested, which may be referred to as A, B, and C. A and C are advertised as remedies for stomach worms, while B, though not advertised on the container for the treatment of stomach worms (*Haemonchus contortus*), has since been recommended by the New Zealand Department of Agriculture for that purpose. Two sheep were treated with A and one sheep each with B and C. One of the sheep treated with A passed four nodular worms (*Oesophagostomum columbianum*). This sheep and the one treated with B died the day after treatment. The other sheep passed no worms.

Of the two sheep receiving A, the one which died had 422 stomach worms (*Haemonchntortus*) *us co*and numerous intestinal nematodes. The other sheep killed four days after drenching had no stomach worms post-mortem and few intestinal nematodes. As this sheep was evidently uninfested with stomach worms (*H. contortus*), it must be left out of consideration. The sheep treated with C passed no worms and had 8 stomach worms (*H. contortus*) post-mortem.

A second trial was made of A on two sheep, the dose given being the same as in the previous experiment—1 tablespoonful of the product (14.8 mils) to 1 pint (473 mils) of milk, as advertised on the label. The sheep passed no worms, and at post-mortem examination were found lightly infested with stomach worms and intestinal nematodes.

While the experiment is inconclusive in regard to the efficacy of coal-tar phenols for stomach worms in sheep, since the experiment animals were so lightly infested, it failed completely as a remedy for lightly infested animals, and there seems to be no reason for considering that it would be more successful in heavily infested sheep.

There can be little doubt that the treatment is dangerous. Two out of four sheep in the first experiment died, the post-mortem showing lesions of pneumonia, pleurisy, and gastritis. In the second experiment both sheep collapsed after drenching, but seemed to recover. The pneumonic condition observed in the dead sheep may be attributed to getting some of the fluid in the lungs, an error in drenching, to be sure, but one almost impossible to avoid when giving as much as 473 mils of drench. In the writers' experience sheep will usually take quietly 118 to 177 mils of fluid by drench; but, after more is given, they begin to struggle, making the drenching increasingly difficult. Furthermore, the hemorrhagic lesions in the stomach of one of the dead sheep would seem to indicate the absorption of phenols through the gastric mucosa.

For worms in dogs.—A further test of the anthelmintic efficacy of phenols was made, using another preparation, which is recommended as an anthelmintic for worms in dogs, the dose recommended being 5 to 8 drops (0.3 to 0.48 mil) in a tablespoonful (14.79 mils) of castor oil. The writers used 0.48-mil doses, administering this dose to each of two dogs, weighing, respectively, 8.6 and 11.3 kgm. On the fourth day after treatment one dog passed two ascarids and the other passed a headless chain of segments of *Taenia hydatigena*. This latter dog showed an infestation with ascarids (*Belascaris marginata*), hookworms (*Ancylostoma caninum*), and tapeworms (*Taenia* sp.), and, as the treatment had been unsuccessful except for the partial removal of a tapeworm, the animal was not killed. The other dog was killed and found to have three *B. marginata* and three *A. caninum*. The treatment, therefore, was somewhat efficacious against ascarids in one case, but entirely inefficacious in the other. It was effective against *A. caninum* in both dogs. It also appears to be ineffective against Taenia, since it did not bring away the head, but this is a point that should have been confirmed post-mortem.

We may conclude from the experiments on dogs and sheep, above recorded, that the phenols in the form of commercial disinfectants and dips are likely to be of little value and dangerous as anthelmintics.

EXPERIMENTS WITH VEGETABLE ANTHELMINTICS

OLEORESIN ASPIDII

For worms in dogs.—*Oleoresin aspidii* is the classic remedy for use against tapeworm. In the discussion of chloroform as an anthelmintic for hookworms (page 403) the writers have already

shown that male-fern in a combination known as Hermann's mixture is efficacious against *Dipylidium caninum* and may have some slight value against whipworms (*Trichuris depressiuscula*) and hookworms (*Ancylostoma caninum*), although the efficacy of the mixture for hookworms is largely due to the chloroform contained in it. The following experiments in which male-fern alone was used corroborate these conclusions.

In the first experimental test of the drug five dogs were used ranging in weight from 6.4 to 15.9 kilos. The treatment was preceded by calomel (65 to 194 mgm., according to the weight of the animals) the afternoon of the preceding day and followed about 45 minutes after treatment by Epsom salt in molasses. One to three mils of male-fern were administered. Within an hour after treatment one dog had passed a mass of fragments of *Dipylidium caninum*, including at least four heads, and another some chains of segments of *Taenia* sp. When the dogs were killed, no specimens of *Taenia* sp. were found, and only two specimens of *D. caninum*, one of which was in the colon, evidently about to pass out. It is evident that the remedy was entirely efficacious against *Taenia* sp., since the dog which was observed to pass Taenia segments after dosing with male-fern was found uninfested on post-mortem examination. It was slightly less efficacious for *D. caninum*, but apparently removed all but one specimen. It is possible that the male-fern removed all individuals of *D. caninum* not attached by burrowing into the intestinal mucosa, leaving embedded heads to renew strobila. This would account for the failure to find more than four heads in the relatively large mass of segments passed.

Male-fern had but little effect on ascarids (*Belascaris marginata*) and removed only one-fourth of the hookworms (*Ancylostoma caninum*) present, in spite of the fact that at one time it was regarded as an appropriate remedy for hookworms (*A. duodenale*) in man and much used for the purpose. It should be stated, however, that, in view of the small number of hookworms involved in this experiment, only four being present, the conclusion that male-fern is inefficacious against hookworms is hardly warranted. None of the three whipworms (*Trichuris depressiuscula*) present were removed by this drug, although Miller (*1904*) reported the successful removal of whipworms from dogs with it.

A further test of the efficacy of oleoresin of aspidium against tapeworms in dogs was conducted by the junior writer. After the usual 24-hour fast, two dogs weighing 20.4 and 11.34 kgm. were given 2.7 and 1.8 mils, respectively, of male-fern, followed by 162 mgm. of calomel.

Prior to treatment the smaller dog had been repeatedly seen to pass chains of proglottides identified as *Taenia pisiformis*, while only a few Taenia eggs had been seen in the feces of the larger dog. The presence of these eggs may have resulted from contamination of the specimen.

Following the anthelmintic the smaller dog passed a few chains of proglottides the first day after treatment, and on the second day 17 fragments and 4 heads. No tapeworms were passed by the larger dog during the four days following treatment, and no nematodes were passed by either dog. The feces of the larger dog were examined for Taenia eggs four days after administering the vermifuge, and were found negative. As this dog had passed no Taenia, and as the original presence of tapeworms was somewhat doubtful, it was dropped from the experiment. The post-mortem examination of the smaller dog showed no Taenia, 15 hookworms, and 13 whipworms. The remedy was therefore entirely efficacious for species of Taenia and entirely inefficacious for hookworms (*Ancylostoma caninum*) and whipworms (*Trichuris depressiuscula*), thus confirming the opinion derived from the previous experiment, that male-fern is very efficacious for tapeworms in dogs and inefficacious for nematodes.

For worms in cats.—Six cats which had been fed with *Cysticercus fasciolaris* formed the subject of this experiment. Each cat was given 0.8 mil of oleoresin of aspidium, followed by 130 mgm. of calomel for the four largest cats and 97 mgm. of calomel in the case of the two smaller cats. Within half an hour one of the cats, which was weak from confinement in a cage and which was suffering from coryza, had died. The post-mortem examination revealed an intense congestion of the gastric mucosa. The characteristic odor of male-fern was noticeable in the stomach, but not in the intestines. A half-grown individual of *Taenia taeniaeformis* and several ascarids (*Belascaris cati*) were found in the small intestine, while three dead *B. cati* were in the colon. Another cat, which was weak from a previous infestation with mange, passed several chains of *T. taeniaeformis* shortly after the administration of the male-fern and died three days later. A third cat vomited one *T. taeniaeformis* and one *B. cati* and still had one *B. cati* post-mortem. Two of the six cats were uninfested with *Taenia* spp., since they passed no tapeworms and none were found post-mortem. Of the four infested animals two died from the treatment, but three of the four were entirely freed from their Taenia infestation, the fourth dying before the remedy had an opportunity to reach the parasite.

It would appear from the above that male-fern is efficacious in removing tapeworms from cats, but that it is apparently more toxic in the case of cats than with dogs, and should only be prescribed for healthy animals. Probably some other taeniacide could be used which might prove as effective and less dangerous. It should be noted that the dose of male-fern given was less than the minimum dose 15 minims (0.9 mil), recommended by Winslow (*1913*). With the exception of the one ascarid, which was vomited, the treatment had no effect on the few ascarids (*Belascaris cati*) and hookworms (*Ancylostoma caninum*) which were present.

PELLETIERINE TANNATE

FOR WORMS IN CATS.—Pelletierine tannate has long been recognized as an effective taeniacide. According to the U. S. Dispensatory —[1]

> The efficacy of pelletierine as a taeniacide has been abundantly confirmed, and it appears to be established that the tannate is the most effective and the least dangerous form of the remedy,—probably because its insolubility prevents its rapid absorption and enables it to come in prolonged contact with the worm.

A test of this drug was made by the junior writer on two cats weighing approximately 3 and 4 kilos each. Sixty-five mgm. of pelletierine tannate per kilo were administered in capsules to each animal and were followed one hour later by 25 mils of castor oil. Prior to the administration of the anthelmintic the cats were starved for 24 hours.

The animals were fed shortly after the administration of the castor oil.

All feces passed during the four days following the administration of the drug were negative for parasites. Cat 2 vomited immediately after feeding, or a little more than an hour after taking the pelletierine. On post-mortem examination cat 1 had 29 *Belascaris cati* and 4 *Dipylidium caninum;* cat 2 had 1 *Taenia taeniaeformis*.

In this experiment pelletierine tannate proved unsuccessful as an anthelmintic for species of Taenia, Dipylidium, or ascarids in cats. The complete failure of the drug is not easy to understand. In the case of cat 2 the drug might have been expelled in the vomitus before it had acted on the Taenia, but this is unlikely, since the vomiting did not occur until more than an hour after the ingestion of the pelletierine.

As the pelletierine tannate used had been in the laboratory for at least six years it was submitted for examination to the Biochemic Division of the Bureau of Animal Industry. The report was returned that the sample responded to all the tests for pelletierine tannate given in the Pharmacopeia. The Pharmacopeia specifies that the drug shall be kept in small well-stoppered bottles away from the light, a condition complied with in this case. There seems to be no reason to suppose that the pelletierine tannate that was used had undergone deterioration, and its failure to give results remains unexplained.

FOR WORMS IN DOGS.—As pelletierine tannate had shown a surprising inefficacy in regard to tapeworms in cats, a further test of this drug was made by the junior writer, using dogs as experiment animals.

The drug was administered to three dogs weighing from 11.8 to 14 kilos, at the rate of 16 mgm. per kilo and was followed one hour later by castor oil. Previous to the treatment 35 mils of castor oil were given to each dog as a preliminary purgative, and the animals were then fasted for 24 hours.

[1] WOOD, G. B., and BACHE, Franklin. THE DISPENSATORY OF THE UNITED STATES OF AMERICA. ed. 19. p. 690. Philadelphia and London, 1907.

Following the administration of the preliminary purgative, two dogs each passed two Taenia proglottides. Following the dose of pelletierine tannate and castor oil, one dog passed five Taenia proglottides, and another dog passed two proglottides. No tapeworm heads or nematodes were found in the feces. The post-mortem examination revealed a few nematodes and *Dipylidium caninum*, but no *Taenia* sp.

It is evident that all three dogs were infested with species of Taenia, since proglottides were recovered in the feces of all animals. The fact that all dogs were free from Taenia on post-mortem examination is indicative of the efficacy of the drug as a taeniacide. The failure to recover the worms in the feces probably resulted from neglect on the part of the attendant collecting the feces.

Pelletierine tannate apparently proved entirely inefficacious against *Dipylidium caninum* or intestinal nematodes in dogs, since none of these parasites was found in the feces, but some were present postmortem. As there is a strong probability that all feces were not collected, the writers can not be certain with regard to the total inefficacy of the drug for these parasites.

The sample of pelletierine tannate used in this experiment was purchased from the same manufacturer and at the same time as the sample used in the previous experiment on cats. It may therefore be assumed to have the same potency as the sample used in the previous experiment, which was tested for purity by the Biochemic Division.

ARECA NUT

FOR WORMS IN DOGS.—Areca nut (*Areca catechu*) is not infrequently prescribed as an anthelmintic for ascarids in dogs. Railliet (*1915*) gives the dose of areca nut as 2 to 4 gm. combined with 10 to 20 gm. of soluble cream of tartar,[1] the latter presumably being used merely as a vehicle. In this experiment 6 gm. of areca nut and 36 gm. of soluble cream of tartar were formed into 12 pills, 4 of which were given to each of three puppies weighing between 1.8 and 2.3 kgm. Previous to the experiment the dogs were each given 14.79 mils of castor oil, which failed to remove any worms, and were then starved for 24 hours. All the dogs were infested with ascarids (*Belascaris marginata*) and hookworms (*Ancylostoma caninum*), as shown by previous fecal examinations. The day following the administration of the vermifuge one of the dogs passed 4 *B. marginata* and was found dead the following morning. The other dogs passed no worms. The post-mortem of the dog that died showed hemorrhagic areas in the colon and feces stained with blood. The other organs were

[1] Soluble cream of tartar, a preparation seldom used by American veterinarians, is boro-tartrate of potassium, made by boiling together four parts of cream of tartar with one part of boric acid in a large amount of water. When most of the water has evaporated, the process of evaporation is completed in a drying oven and the resultant salt is pulverized and stored in well-stoppered bottles. Soluble cream of tartar is very deliquescent, and hence well adapted for making a pill mass.

entirely normal in appearance. This puppy had 23 ascarids (*B. marginata*) and a few hookworms (*A. caninum*) and whipworms (*Trichuris depressiuscula*). The other dogs had varying numbers of ascarids, hookworms, and whipworms. The treatment removed only 4 out of 67 ascarids and had no effect on the hookworms and whipworms present. It should be stated in comment that the areca nut used was ground 14 months previous to the experiment and hence may have lost some of its potency. This leaves open the question as to the efficacy of freshly ground areca nut, but it appears from the experiment that areca nut ground one year previously is not a very efficacious anthelmintic for dogs, and may possibly cause serious digestive disturbances. While it is not possible to determine whether the colitis noted in the dead dog resulted from the drug, it should be stated that this puppy had been weak and emaciated for some time previously and was in poor condition to undergo anthelmintic treatment.

For worms in poultry.—Areca nut is quite commonly prescribed as a remedy for tapeworms in poultry. The following experiment was made to test its efficacy against tapeworms and incidental nematode parasites of the intestinal tract of chickens:

One gm. of powdered areca nut was given to each of six chickens, weighing about 453 gm. each, the dose being given in 4 mils of olive oil. Prior to the experiment the birds were fasted for 24 hours.

The total number of worms passed following the vermifuge were 20 *Heterakis papillosa*, which were passed by three of the six birds on the first day, and some tapeworm segments, including at least one head with these. On post-mortem examination large numbers of tapeworms and *H. papillosa* were found, besides other nematodes, which, from their location in the esophagus and proventriculus, would not be likely to be affected by anthelmintics. Probably more tapeworms were passed than were counted, since, when a small tapeworm head is passed and then given an opportunity to dry, it is very difficult to detect even by a careful examination of the feces. But at best the drug seems to have had very little efficacy against *H. papillosa* or tapeworms.

The areca nut used in this experiment was ground at least four years previously. This leaves open the question as to the efficacy of the freshly ground product. If it is true that the drug loses its potency after grinding, this constitutes a serious objection to its use in commercial products, since it is unlikely that it will be freshly ground when purchased.

SANTONIN

Santonin is the classic remedy for ascarids and forms the basis of most of the worm remedies for children. It has also been widely used and recommended in canine practice. As this drug is largely of Euro-

pean or Asiatic origin, the present price of santonin is almost prohibitive in veterinary medicine, and its place is being taken by the native and, in the writers' experience, more effective drug chenopodium. Santonin is usually combined with or followed by a purgative to promote elimination of the parasite. For this purpose calomel or areca nut is much used.

SANTONIN AND CALOMEL FOR WORMS IN DOGS.—The dose of santonin for dogs recommended by Winslow (*1913*) is from 1 to 3 grains (65 to 195 mgm.). Taking 130 mgm. as the dose for an average dog weighing 10 kgm., the writers gave seven dogs, ranging in weight from 3.8 to 9.5 kgm., doses graded from 32 to 130 mgm., accompanied by the same amount of calomel. The treatment was preceded by the administration of castor oil, 29.57 mils to dogs weighing 4.5 to 9.5 kgm., and 7.39 mils to pups under 4.5 kgm. Food was withheld the previous day. Seven ascarids (*Belascaris marginata*) were passed following the administration of castor oil and 13 *B. marginata* and two whipworms (*Trichuris depressiuscula*) after santonin and calomel. At the post-mortem examination it was found that about one-fourth of the ascarids remaining after the action of the castor oil later had been removed by santonin and calomel. The treatment was very inefficacious for whipworms, removing 2 out of 72, and entirely inefficacious for *Dipylidium caninum* and Taenia.

It would appear from the foregoing that santonin and calomel, the remedy usually prescribed for ascarids, is not very efficacious for dogs in single doses.

As the efficacy of the santonin as shown by the above experiment was considerably less than had been expected, considering the well-established reputation that santonin has as an ascaricide, a second experiment was undertaken to determine the efficacy of santonin and calomel in repeated doses. In this experiment four dogs, weighing from 1.8 to 9 kilos, were given graded doses of equal quantities of santonin and calomel, the dose varying from 32 to 130 mgm., according to weight. The first dose was given after a preliminary fast, and the second dose was given two days after the first, food being withheld the evening before the second treatment also, the same dose being given at each treatment. The first treatment was preceded by castor oil, which failed to eliminate any worms. One of the dogs passed no ascarids and had none post-mortem, so that it was evidently not infested and must be left out of consideration. From the three remaining dogs it eliminated 7 out of 10 ascarids, a distinct gain in efficacy compared with the previous experiment, in which a single dose was given. In this case the drug also showed a fair degree of efficacy against whipworms (*Trichuris depressiuscula*), parasites which in the writers' experience are difficult to remove with any degree of certainty with any of the anthelmintics tested. The drug was entirely ineffective against hookworms and *Dipylidium caninum*.

Santonin and calomel for worms in hogs.—Before the present European war, when the price of santonin justified its use for live stock, it was much recommended for roundworms (*Ascaris suum*) in swine. Since the separate dosing of a herd of swine requires considerable time and labor and the handling of the animals is likely to excite them and may lead to their being injured, it is usually the custom to mix the medicine with the feed, allowing a few hogs to eat from the same trough at one time. If the drug is well mixed with the feed and the animals are of about the same size, it is assumed that they will get approximately the same dose. This method was tested by the junior writer for a number of drugs. In this series of experiments the worms passed were not counted; nor were the animals killed. The feces were previously examined for ascarid eggs, and the efficacy of the drug was judged solely by the presence or absence of eggs in the feces three days after the administration of the anthelmintic, care being taken to verify all negative findings by one or more repeated fecal examinations at 3-day intervals.

In the first experiment, after a preliminary fast of 24 hours, three hogs weighing 15.4 to 18 kgm. were given santonin and calomel allowing 130 mgm. of each per hog. The drug was shaken up in 473 mils of water, poured over a bran-mash feed and thoroughly stirred in. The feces of all the pigs were positive for ascarids (*Ascaris suum* or *suilla*) at the beginning of the experiment. Three days after the treatment the feces of all the pigs were still positive. As the treatment failed to free any of the pigs from ascarids (*A. suum*), it was repeated, giving 194 mgm. of santonin and calomel instead of 130 mgm. The second treatment was equally without results. A third trial was made in which the pigs were dosed individually after preliminary starvation, each pig receiving 194 mgm. each of santonin and calomel. This treatment resulted in the apparent elimination of all ascarids from one of the pigs, the other two remaining infested. These hogs were then dosed individually with 259 mgm. each of santonin and calomel, all subsequent fecal examinations being negative.

It appears from the above that santonin and calomel should be given in repeated and separate doses to be effective. Probably three doses of santonin at intervals of three days would be effective. No symptoms of poisoning were observed either in dogs or pigs, although the pigs were given a dose more than twice as large as is usually prescribed for animals of their size. According to the Veterinary College at Ames, Iowa,[1] not over 4 grains (259 mgm.) of santonin should be given to the largest hog. It would seem that the treatment of swine with santonin requiring apparently three separate doses to be effective, is far too laborious and costly for use, especially as we have a more reliable and less expensive drug in oil of chenopodium.

[1] Treating pigs for worms. *In* Breeder's Gaz., v. 64, no. 8, p. 315. 1913.

SANTONIN AND ARECA NUT FOR WORMS IN DOGS.—Areca nut is frequently prescribed with santonin, since it furnishes the necessary laxative, and having anthelmintic properties of its own is supposed to be an adjuvant to the santonin. In this experiment four dogs weighing from 2.7 to 6.8 kgm. were given 33 to 130 mgm. of santonin combined with 0.78 to 1.94 gm. of areca nut. The dogs were given a preliminary dose of castor oil which removed 7 ascarids (*Belascaris marginata*). Following the administration of the santonin and areca nut 3 *B. marginata* were passed. At the post-mortem examination 30 ascarids (*B. marginata*) were found besides several hookworms (*Ancylostoma caninum*), whipworms (*Trichuris depressiuscula*), and 1 *Dipylidium caninum*. One of the dogs vomited the santonin, which may account for its failure to act in this case. In this case santonin proved less efficacious for ascarids than castor oil, and had no effect on the other intestinal worms present.

SANTONIN AND ARECA NUT FOR WORMS IN HOGS.—Combinations of santonin and areca nut are frequently prescribed for pigs, to be mixed with the feed. The following, copied from the Breeder's Gazette, is typical:

Santonin	2½ grains.
Areca nut	1 dram.
Calomel	½ grain.
Sodium bicarbonate	½ dram.

This quantity is considered sufficient for a 100-pound hog. As the pigs experimented with weighed only 24.5 kgm. each, two-thirds of the amount prescribed was allowed for each pig and mixed with the feed, after starving the animals 24 hours. Three days after the experiment the feces were still positive for *Ascaris suum*, and a second trial was made using the full amount prescribed in the formula. This experiment was also unsuccessful in ridding any of the hogs of ascarids. A third trial of the formula was made on a pig weighing 24.5 kgm. with the object of testing the efficacy of repeated doses. Five times the amount prescribed was made up and divided into seven powders, one powder being mixed with the feed every morning for seven consecutive days. At the end of the period no diminution in the number of ascarid eggs in the feces was discernible.

As far as these experiments can be analyzed, it appears that santonin and areca nut offer no advantages over santonin and calomel, and that either combination must be repeated and given in individual doses on an empty stomach to be efficacious. The last experiment illustrates the inadvisability of giving drugs to hogs, mixed with the feed, since in this case, a drug already shown to be efficacious in repeated doses was apparently a complete failure when given in this manner.

THYMOL

For worms in dogs.—Prior to the beginning of the great European war, thymol was the classic remedy for hookworm in man, and it was only after the war had made this drug practically unobtainable that serious efforts were made to find a substitute for it. In a general way it was regarded as a satisfactory drug, and we expected to find a fairly high coefficient of efficacy for it in our experiments.

In our first experiment thymol was administered at the rate of 130 mgm. per kilo of body weight, giving the thymol in capsules to 2 dogs and in drench to 2 dogs; in the latter case the thymol was dissolved in a small amount of alcohol and this added to water to make a fine suspension. After an interval of a half hour to an hour, calomel in doses of about 65 mgm. per 2.5 kilos of weight of dog was given. All dogs were fasted from the previous day.

As regards hookworms, thymol removed 15 out of a total of 114 worms present, being entirely ineffective in the case of three dogs, two of which received the thymol in capsule and one of which received it in aqueous suspension. In one dog, however, it removed 15 out of 16 hookworms.

In regard to ascarids, thymol removed nearly three-fourths of these worms present, a very satisfactory showing considering that thymol is not usually considered especially valuable as an ascaricide. It was not efficacious when administered in aqueous suspension. Thymol also exhibited some efficacy against whipworms and was very slightly effective for *Dipylidium caninum.*

In the above experiment thymol showed a low degree of efficacy against hookworms, the parasite for which it is usually prescribed, and was more efficacious against whipworms and still more efficacious against ascarids. In the experience of the writers a number of drugs were found to be occasionally efficacious against whipworms, while at other times they were decidedly inefficacious. This variation may perhaps be explained as a matter of accident, depending on whether or not the drug succeeds in penetrating to the cecum in its passage through the alimentary tract.

An objection to thymol at present is its cost and scarcity.

In order to determine whether the low degree of efficacy displayed against hookworms by thymol was due to the mode of administration, the experiment was repeated. This time the animals were subjected to preliminary purgation with Epsom salt, and the administration of calomel after treatment was delayed for five to six hours. The thymol was given in the same dosage, 130 mgm. per kilo of body weight, and was given in aqueous suspension.

Three dogs weighing 4 to 9.3 kilos were used for the experiment. They were given Epsom salt at the rate of 3.7 mils for each 5 kilos of weight, the day prior to the administration of the anthelmintic, and five to six

hours after giving thymol they were given calomel at the rate of 65 mgm. for each 2.5 kilos of body weight.

One of the dogs died immediately after receiving the thymol, and the remaining two dogs each passed one ascarid. The post-mortem examination showed that one or both dogs were infested with hookworms (*Ancylostoma caninum*), whipworms (*Trichuris depyessiuscula*), ascarids (*Belascaris marginata*), and *Dipylidium caninum*. The treatment, therefore, proved an entire failure for all worms present except ascarids, for which it showed a rather low degree of efficacy in this experiment.

As the thymol used had not proved as efficacious against hookworms as might be expected, a sample was sent to the Biochemic Division of the Bureau for analysis. An analysis was made by Dr. Custis, who reported that it responded to all tests for thymol and showed no impurities. Thymol is apparently a stable phenol; the writers are unaware of any evidence indicating that it deteriorates.

Since thymol in a single dose proved so ineffective in the foregoing experiments, an experiment was undertaken to determine its efficacy in repeated doses. Four dogs weighing 2.3 to 3.9 kilos were given a preliminary purgation with 20 mils of castor oil on the day previous to treatment and were then fasted for 24 hours. Thymol was given at the rate of 130 mgm. per kilo of body weight, and this dose repeated three and five days later for a total of three doses. After the second and third doses of thymol, 97 to 130 mgm. of calomel were given. The first dose of thymol was given dissolved in alcohol to one dog and resulted in the immediate death of the animal. The other dogs were given the thymol in aqueous suspension.

The thymol removed 8 out of the 16 hookworms (*Ancylostoma caninum*) present in the dogs, a rather poor showing in view of the preliminary fasting, purgation, and repeated doses, but decidedly better than in the previous experiments. It removed the only ascarid (*Belascaris marginata*) present and showed some slight degree of efficacy against whipworms (*Trichuris depressiuscula*).

It appears from these three experiments that thymol to be at all successful as an anthelmintic for hookworms (*Ancylostoma caninum*) must be preceded by purgation and fasting and given in repeated doses. At least two such courses of treatment should be given to remove the greater number of the worms present, and further treatment combined with prophylaxis is desirable. As a matter of fact, this is the usual procedure in administering thymol in human practice, and it is usually realized by physicians that it is very difficult to remove all the hookworms present, as it is observed that hookworm eggs often persist in the feces after repeated thymol treatments.

In the three experiments considered together, thymol removed over half the ascarids present, but showed very little efficacy for whipworms or

Dipylidium caninum. That the administration of thymol to dogs is not without danger is shown by the death of 2 out of the 11 experiment animals.

The low efficacy and the danger in the use of thymol do not compare well with the comparatively high efficacy and safety in the use of chloroform, so far as the experiments with dogs are concerned. While the writers are impressed with the dangers in connection with the administration of most anthelmintics, and these dangers are quite impressive for chloroform as well as thymol, nevertheless, it seems that, with the exceptions already noted in the discussion of chloroform for cases where there is heart trouble, or lesions of the parenchymatous viscera, chloroform is not only much more effective than thymol, but, in therapeutic doses, is safer.

TURPENTINE

Turpentine is a remedy very commonly advocated for use against nematode parasites, especially those in chickens, horses, and swine. The obvious objection to its use is its well-known injurious effect on the kidneys.

FOR WORMS IN POULTRY.—Since the treatment of chickens for worms is for obvious reasons so seldom undertaken by veterinarians and so commonly by owners of poultry who are not especially trained in medical lines, it is not surprising that the dose advocated for use in this connection is very variable. Some writers recommend a half teaspoonful of turpentine in an equal amount of olive oil; others recommend 1 to 3 teaspoonfuls of turpentine undiluted.

An experimental test of the efficacy of the lighter dose of turpentine against worms in poultry was made as follows: Six chickens weighing between 0.45 and 0.9 kgm. were given 2 mils of turpentine mixed with 2 mils of olive oil, the birds being fasted from the previous day and the dose being followed at once with 8 mils of castor oil. About five hours after treatment all the birds had passed some feces, the feces having an odor of turpentine.

The treatment appeared to be fairly satisfactory for the large roundworm (*Ascaridia perspicillum*) in the small intestine of chickens, since it removed more than three-fourths of the worms present, as shown by post-morten examination. It had little effect in cases of infestation with large numbers of cecum worms (*Heterakis papillosa*) with which chicks are frequently infested. According to the experience of the writers, this worm is difficult to remove with any anthelmintic, since its location protects it to a greater or less extent from contact with the drug.

Turpentine was equally inefficacious as a remedy for tapeworms in fowls, removing only 8 out of 444. It should be stated, however, that it is very difficult to count the tapeworm heads which may be present in the

feces. On account of their minute size they dry quickly, and unless the feces are examined very soon after they are passed, many specimens will be unrecognized. The remedy, therefore, may have been more effective for tapeworms than the figures indicate. Its principal advantage, however, seems to be as a remedy for roundworms (*Ascaridia perspicillum*).

For worms in dogs.—Four dogs weighing 2.5 to 7.7 kilos were given preliminary purgation with castor oil, and dosed with turpentine at the rate of 1 mil per kilo of weight. The turpentine was given mixed with castor oil, 15 to 30 mils, according to the weight of the animals.

The preliminary purgation with castor oil did not bring away any worms. One of the dogs died on the fifth day after treatment.

Only one worm, an ascarid, was passed; to this should be added one ascarid found in the rectum of the dog that died, making two ascarids removed by the turpentine.

In view of the lack of results from the treatment, the dogs in this experiment were not killed. Just previous to the experiment, microscopic examination of the feces for eggs had shown that all of the dogs were infested with either hookworms or ascarids or both. On the last day of the experiment the feces were again examined for eggs, and eggs of the same worms were found to be still present.

All the dogs showed symptoms of distress immediately after the treatment with turpentine, the principal feature being a temporary paralysis of the hind quarters.

It may be concluded that turpentine in doses of 1 mil per kilo of weight is not very efficacious against ascarids in dogs, is entirely inefficacious against hookworms, gives rise to pronounced suffering and temporary paralysis of the hindquarters, and may kill the dog.

For worms in hogs.—Turpentine is frequently prescribed as a remedy for roundworms (*Ascaris suum*) in swine. It is often given in repeated doses mixed with the feed. A better way is to make an emulsion of the turpentine with equal parts of flaxseed decoction. This is more easily miscible with the feed, and avoids the burning caused by the ingestion of pure turpentine.

An emulsion of turpentine and flaxseed decoction, made by boiling 85 gm. of flaxseed in 296 mils (10 ounces) of water, straining, and adding an equal amount of turpentine, was fed to three hogs by the junior writer. The hogs weighed from 45.36 to 68.04 kgm. The equivalent of 7.4 mils of turpentine per hog or 44.36 mils of the mixture, was mixed daily in the feed of the three hogs for seven days. At the end of seven days all the hogs were listless, refused to eat, and were constantly voiding small amounts of urine, the nephritic symptoms continuing for a week after the treatment stopped. All the feces of the hogs contained ascarid (*Ascaris suum*) eggs after the experiment was concluded.

The treatment was decidedly inefficaceous and highly dangerous. The animals never gained as rapidly as the other hogs kept under the same conditions but not dosed with turpentine. While separate dosing might have shown some efficacy for the treatment, the injury to the hogs precludes its recommendation as an anthelmintic.

FICUS LAURIFOLIA

For worms in dogs.—The latex of *Ficus laurifolia* has been highly recommended by Berrio (*1911*) and by Mouat-Biggs (*1915*) for use in expelling whipworms from man, and is also said to have been adopted by the Venezuela State Board of Health as the official remedy for use against hookworms. Berrio gives doses of 25 to 30 gm., followed by castor oil. Mouat-Biggs gives the latex morning, noon, and night, mixed with water, which may be sweetened, or with milk, in doses of 10 to 40 gm., according to the age of the patient.

A sample of the expressed juice of the latex kindly supplied to the writers by Dr. Gonzalez-Rincones of Caracas, Venezuela, was tested on three dogs weighing from 4 to 29 kilos. Fifteen to 30 mils of the juice were given, preceded by a purge of 30 mils of castor oil, and followed three or four hours after the administration of the latex by 15 to 30 mils of castor oil. Although all the dogs were infested with hookworms, whipworms, or ascarids, as shown by a previous fecal examination, no parasites were passed. Since the remedy was entirely inefficacious, the dogs were not killed.

The sample forwarded to the writers did not conform to the published accounts of the latex, which describe it as of thick, sirupy consistency and milky white in color, but was instead a watery fluid with something resembling curds floating in it.

A second sample conforming in all respects to the descriptions of the latex was subsequently received through the courtesy of Dr. Rincones.

This drug was tested by the junior writer on three dogs weighing 4.5 to 18.2 kilos. The latex was given at the rate of 2.6 mils per kilo of weight and followed by castor oil at the rate of 3 mils per kilo of weight. The treatment was preceded by a dose of castor oil the day before, and the animals were starved for 24 hours. The drug removed 9 out of a total of 11 ascarids (*Belascaris marginata* and *Toxascaris limbata*), 1 out of 33 hookworms (*Ancylostoma caninum*), and 1 out of 50 whipworms (*Trichuris depressiuscula*), thus showing but little anthelmintic value for the two worms (hookworms and whipworms) for which it is particularly recommended, and a very satisfactory anthelmintic efficacy for ascarids. It was ineffective against *Dipylidium caninum*, no specimens being passed and several being found at autopsy. Of the three nematodes on which the latex had some effect, ascarids are, according to the experience of the writers, most easily removed—probably because they do not attach

themselves to the mucosa like hookworms and whipworms. The writers have usually found that any drug which is at all efficacious against hookworms is equally so against ascarids.

Evidently the latex has definite anthelmintic properties, and although its efficacy against hookworms (*Ancylostoma caninum*) and whipworms (*Trichuris depressiuscula*) was very slight in this test, it is worthy of further experimentation in repeated dosage. As the liquid expressed from the latex appears to have no value as a vermifuge, the active principle may be considered to reside in the solid portion sustained in the latex in the form of an emulsion.

SPIGELIA

FOR WORMS IN DOGS.—Spigelia, or pinkroot, is the dried rhizome and roots of *Spigelia marilandica*, a plant native to the Southern and Southwestern States. Its virtues as an anthelmintic are said to have been known to the Cherokee Indians. According to the U. S. Dispensatory, 19th edition,[1] it is generally considered among the most powerful anthelmintics, and is used especially for roundworms. The fluid extract is official.

A test of the fluid extract of Spigelia was made by the junior writer. In this experiment four dogs weighing from 4.8 to 18 kgm., all of which were infested with hookworms, and one with whipworms, were treated with Spigelia in doses graduated from 1.5 to 6 mils, according to the weight of the dogs. The drug was followed by 194 to 324 mgm. of calomel. The dogs were previously dosed with 29.57 mils of castor oil and then starved for 24 hours.

One dog was not killed, as it passed no worms. As the previous fecal examination for this dog showed hookworms, it is evident that the drug was ineffective in this case for hookworms. The three dogs remaining passed altogether 6 hookworms, including 3 found in the rectum postmortem in the process of elimination. There were 199 hookworms left unaffected by the treatment. Spigelia removed 1 out of 18 ascarids and 2 out of 29 whipworms. A few segments of *Dipylidium caninum* were passed, but no heads.

The remedy therefore appears to have but little efficacy as an anthelmintic for any of the common parasites of dogs, but further trial is advisable to determine this point.

TOBACCO

FOR WORMS IN CHICKENS.—Herms and Beach (*1916*) have devised a method of treating poultry for worms, consisting in the administration of chopped tobacco stems and the liquid in which they are steeped.

Finely chopped tobacco stems, 453 gm. or 1 pound, enough for 100 birds, are soaked for two hours in enough water to keep them covered.

[1] WOOD, G. B., and BACHE, Franklin. Op. cit., p. 1162.

Both the stems and water are mixed with half the usual ration of ground feed and given to the fowls. Two hours later one-fourth the usual ration is given mixed with Epsom salt at the rate of 312 gm. for each 100 fowls. The treatment is to be repeated one week later. The cost is said to be only 10 cents for 100 fowls.

In the present experiment six chickens were deprived of food for 24 hours and then fed the tobacco mash; two hours later they were fed the Epsom salt mash in the proportions recommended by Herms and Beach. The next day they were given what was left of the tobacco mash, since they had refused to clean it up the first time. A mixture of bran and tobacco was used, and the birds were not very eager for it, even after the preliminary fasting.

The remedy removed 30 out of 162 *Heterakis papillosa*, 3 out of 39 tapeworms, and had no effect on *Tetrameres* sp., a parasite which, from its location in the mucous glands of the proventriculus, would not be likely to be affected. This treatment is apparently intended especially for *Ascaridia perspicillum*, since the "roundworm" figured in the paper by Herms and Beach (*1916*) is evidently of this species. Unfortunately the efficacy of the treatment for this parasite could not be determined, since no species of Ascaridia were present in the birds used in the experiment.

While the tobacco treatment failed to free any bird from all of the worms of any given species that might be present, nevertheless the treatment seems to have been more successful against *Heterakis papillosa* or against tapeworms than the other treatments tried.

In view of the difficulty of removing *H. papillosa* from the ceca, it may be assumed on the showing here that this treatment would be effective in removing *Ascaridia perspicillum* from the small intestine, especially if the dose is repeated, as recommended by Herms and Beach.

OIL OF CHENOPODIUM

Oil of chenopodium is derived from the distillation of the seeds or of the entire leafy part of *Chenopodium anthelminticum* L., sometimes referred to under the synonym "*Chenopodium ambrosioides anthelminticum* A. Gray," and commonly called "chenopodium," "American wormseed," or "Jerusalem oak." According to Henkel (*1913*)—

Wormseed has been naturalized in this country from tropical America and occurs in waste places from New England to Florida and westward to California.

Infusions made from chenopodium were used in the United States by the early settlers as a treatment for infestation with ascarids in man, and its anthelmintic properties are said to have been known by the Indians. Oil of chenopodium has only recently come into prominence as a result of the shortage of thymol and santonin, for which it has proved an effective substitute.

The chemistry of the oil of chenopodium has been studied by Nelson (*1911, 1913*), who was unable to ascertain its exact chemical nature, but concludes that it is an unstable dioxid. Its physiological action and toxicity have been the object of numerous experiments by Salant et al. (*1915*) and Nelson (*1911, 1913*). They note that it is a respiratory depressant and that it decreases vagus instability and diminishes frequency of heart action.

In regard to its toxicity the minimum lethal dose is twice as great when given by the mouth as when given hypodermatically. Repeated doses have a cumulative effect, and the toxicity is enhanced when the experiment animals are starved. It is less toxic when combined with nonessential oils, such as olive, cottonseed, or coconut oil. In cats 0.6 mil per kilo by mouth was invariably fatal, but in dogs 0.5 mil caused only vomiting. Adrenalin and digitalis were found to be antagonistic to oil of chenopodium.

Heiser (*1915*) notes that over 100,000 cases of hookworm infestation involving both *Ancylostoma duodenale* and *Necator americanus* have been treated in the Orient with chenopodium.

For worms in dogs.—In view of the present great interest in chenopodium, the writers have made a considerable number of tests of this substance with a view to determining its efficacy against worms in dogs and other domestic animals, and in nearly all cases they have found it extremely efficacious, especially for ascarids. The following experiment was conducted to determine the efficacy of oil of chenopodium when administered in one dose at the rate of 0.3 mil per kilo.

Eight dogs, weighing from 2 to 10.3 kilos, were given a preliminary purge of castor oil, which resulted in the removal of 9 ascarids. After starving for 24 hours, they were given chenopodium in the dosage indicated, mixed with 2.5 to 15.5 mils of castor oil, according to weight. The treatment was found extremely efficacious for ascarids, removing 160 worms and leaving only 2. From all but one of the dogs it removed all ascarids, and removed 10 out of 12 from that one. Its effect on hookworms and whipworms was less striking, about one-fourth of the total number present being removed. It had very little efficacy against *Dipylidium caninum*. It was evident that the dose given was unnecessarily large, since the feces smelled strongly of chenopodium, and six of the eight dogs vomited from two to four hours after treatment. An objectionable feature of the chenopodium treatment, as given, was the excessive ptyalism that it caused and the fact that the dogs objected to the taste of chenopodium in the castor oil and resisted its administration.

In the second experiment with chenopodium an attempt was made to overcome the ptyalism by giving the chenopodium in a capsule. Eight dogs, weighing from 5.5 to 9 kilos, were given chenopodium in capsules at the rate of 0.2 mil per kilo on each of three successive days, the drug being followed with five times its volume of olive oil the first two doses,

and five times its volume of castor oil the third dose. While this treatment removed 15 out of 17 ascarids (*Belascaris marginata*), it was ineffective for hookworms (*Ancylostoma caninum*), whipworms (*Trichuris depressiuscula*), and *Dipylidium caninum*. This method was less satisfactory than that employed in the previous experiment. The castor oil given was insufficient to overcome the constipating effect of the chenopodium; the chenopodium, being promptly released from the capsules, was brought undiluted against the gastric mucosa, causing considerable irritation, as shown by the fact that all dogs vomited promptly after the administration of the drug. Two of the dogs died from the treatment, one from traumatic pneumonia, due to the fact that the capsule opened in the larynx, allowing oil to penetrate the lungs. The other dog which died was a bitch, containing 11 well-developed fetuses, and it is likely that the pregnancy in this case was a condition contributing to the animal's inability to withstand the large amount of chenopodium given.

A third experiment was undertaken to test the efficacy of oil of chenopodium when given in doses of 0.1 mil per kilo, and the dose repeated daily for a total of six doses. The chenopodium was given mixed with 10 times its volume of olive oil, and was preceded each day by castor oil in amount equal to the olive oil; 1 minim of chloroform per kilo of weight was added to the mixture of chenopodium and olive oil the first day and to the castor oil on the following days. The animals were kept on half feed during the time the treatment was being given.

In this experiment all of the 8 ascarids present and 94 out of 133 hookworms were removed. This increased efficacy against hookworms is in the writers' opinion due largely to the chloroform administered with the chenopodium, since in none of their numerous experiments with chenopodium have they ever found it so efficacious for hookworms when given with castor oil only. The treatment was entirely ineffective for whipworms (*Trichuris depressiuscula*) or for *Dipylidium caninum*.

This method of administration proved generally satisfactory. The individual dose was sufficiently diluted to prevent any undue ptyalism when administered, and there were no symptoms of acute distress, which occurred when the oil was given in a capsule.

The results of this experiment led the writers to make another test of chenopodium and chloroform as a remedy for mixed infestation, chenopodium being in their experience the most successful remedy for ascarids and chloroform the best remedy for hookworms. This combination seems to be entirely compatible both physiologically and pharmaceutically. These drugs when combined with castor oil form a homogeneous and fairly stable mixture, and the action of each drug is not inhibited by the other.

Four dogs having been given a preliminary purgative of castor oil, followed by a 24-hour fast, were given chenopodium and chloroform, both

at the rate of 0.1 mil per kilo. The drugs were given in castor oil, varying in amount from 12 to 40 mils, according to the weight of the animals. In this case, as usual, the chenopodium proved efficacious against ascarids (*Belascaris marginata*), removing 7 out of 8. The remedy showed an appreciable effect on the whipworms (*Trichuris depressiuscula*), removing about one-fifth of the total number. In regard to hookworms, however, only 7 out of 61 were removed.

In order to ascertain whether the efficacy of the remedy for hookworms would be enhanced by increasing the dose, a second trial was made in which 0.2 mil per kilo, or double the amount of both chenopodium and chloroform, were administered. As in the previous case, the treatment was preceded by the administration of castor oil 24 hours before dosing, and the dogs allowed no food until after the administration of the anthelmintics. The chenopodium and chloroform were given in castor oil, allowing 29.57 mils for each dog. The preliminary purgative removed one ascarid (*B. marginata*) and some segments of *Dipylidium caninum.*

The combination of chenopodium and chloroform in the dosage given proved highly successful. All ascarids and over half the hookworms present were removed. As usual, it was entirely ineffective for *Dipylidium caninum.* Undoubtedly the dosage of chenopodium and chloroform at the rate of 0.1 mil per kilo is too small for the best results, and the efficacy of both drugs is enhanced by doubling the amount. At the same time this increased dose seems to be well within the limits of safety and has been tested several times by the junior writer, always with satisfactory results.

In view of the fact that castor oil is more or less objectionable to many persons and is not well tolerated by others, the writers undertook an experiment in which liquid petrolatum was substituted for castor oil in connection with oil of chenopodium. Four dogs weighing 4.5 to 15 kilos were given chenopodium in doses of 0.2 mil per kilo mixed with 10 mils of liquid petrolatum, the drug being followed at once by 20 mils of liquid petrolatum. The animals were fasted from noon of the previous day. One of the dogs died on the third day following the administration of chenopodium. The treatment was entirely inefficacious for ascarids, hookworms, *Dipylidium* sp., or *Taenia* sp., and removed only 2 of 28 whipworms. While the experiment indicates that the liquid petrolatum diminishes the efficacy of the chenopodium, it must be noted that there was only one ascarid present, and while the treatment should have removed the worm, this is a rather small basis on which to judge the performance of the anthelmintic. On the other hand, the liquid petrolatum seems to be unequal to the task of overcoming the constipation and toxic effects from the chenopodium, as the treatment resulted in the death of one dog.

It is the opinion of the writers that the mechanical lubrication resulting from the use of liquid petrolatum is not sufficient to overcome the constipation and that an active purgative, preferably castor oil, is indicated.

The foregoing experiments with chenopodium and chloroform, in comparison with other standard anthelmintics used for the removal of worms from dogs, indicate that oil of chenopodium is the best anthelmintic of those tried for use against ascarids (*Belascaris marginata* and *Toxascaris limbata*). In the 6 experiments, involving the use of 34 experiment animals, it removed 194 out of 200 ascarids, an efficacy of 97 per cent. It is at the same time probably a little more efficacious against whipworms (*Trichuris depressiuscula*) than any other anthelmintic tested. In the entire six experiments its average percentage of efficacy was only 12, a figure which illustrates the difficulty experienced in dislodging this parasite. Moreover, it is probable that the chloroform used in three of the experiments may be responsible for the removal of some of these worms, since both chloroform and chenopodium were found to have a limited efficacy for whipworms when given separately.

As regards hookworms (*Ancylostoma caninum*), the experiments of the writers with chenopodium do not show as high an average efficacy for this drug as they had been led to expect, considering the warm indorsement chenopodium has frequently received as an anthelmintic for the hookworm of man. Undoubtedly chenopodium as well as thymol would have to be given in repeated doses and the treatment renewed in order to secure a high percentage of efficacy. The writers have found chloroform much more efficacious than chenopodium for hookworms in dogs, and, when given at the rate of 0.2 mil per kilo, is apparently free from danger.

For worms in hogs.—A series of experiments to test oil of chenopodium as an anthelmintic for ascarids in swine were carried out by the junior writer and seemed to indicate the great value of this drug when given to hogs individually after a period of starvation. In these experiments the hogs were not killed, the effect of the anthelmintic being judged by the presence or absence of ascarid eggs in the feces passed subsequent to treatment.

In the first experiment with chenopodium an effort was made to determine the minimum effective dose necessary to free a hog from ascarids. In this case the dose of oil was combined with 3.7 mils of areca nut and mixed with the feed, after starving the pigs. Each pig was fed separately, thus assuring full dosage to each animal. Two pigs, weighing 11.32 and 14.06 kgm., respectively, were given 0.2 mil of chenopodium, while a third pig weighing 37.9 kgm. received 0.4 mil. This latter pig was apparently free from ascarids after a single dose, while the two other pigs each received three additional doses of 0.27 mil, 0.36 mil, and 0.54 mil before their feces were negative for ascarid eggs.

It is evident from the above that, even when given mixed with feed, a method later shown to be unsuitable, a relatively small dose of oil of chenopodium may prove efficacious. It should be stated in comment that at the time these experiments were conducted there was little

modern literature in regard to the use of oil of chenopodium, and, so far as the writer is aware, no use had been made of the oil as an anthelmintic for swine. Hence, the drug was administered cautiously. Subsequent experiments have shown that doses of chenopodium relatively many times larger are perfectly well tolerated by swine and are more certain in action.

In a second experiment with swine chenopodium was administered in castor oil, and was not given mixed with feed but was fed with a spoon to each hog individually. Four of the five hogs used were each given 28.35 gm. of Epsom salt mixed with their feed, while the fifth hog was given none. All animals were then starved for 24 hours before the administration of the chenopodium. The smallest pig, weighing 12.25 kgm. was given 0.8 mil; the others, weighing 12.7 to 28.12 kgm. were given 1 mil. The dose of chenopodium in each case was given with 29.57 mils of castor oil. This treatment was entirely successful. All five hogs had shown numbers of ascarid eggs in their feces prior to the treatment, and all were found free from ascarid eggs a week later. The feces of the hogs were again examined three days later and found negative. Two of the five hogs received a third fecal examination, which was consistently negative.

It appears from the above that chenopodium is an excellent anthelmintic when given in individual doses after preliminary starvation, and in most cases may be relied on to remove all the ascarid worms present. This opinion was confirmed by further experiments. The preliminary dosing with Epsom salt did not seem to be of any benefit, since the chenopodium was as successful in the case of the hog which received no salt as in the case of those that did. Subsequent experience has shown that in the chenopodium treatment for hogs preliminary purgation is an unnecessary expense.

As there is always a demand among stockmen for something that can be mixed with the feed to rid their hogs of worms, an experiment was undertaken to determine the possibilities of this form of medication. As hogs, especially if large, are very difficult to handle and as much time and effort would be required to dose separately each animal of a large herd, the advantage of mixing the medicine in the feed is obvious. Unfortunately this method proved impracticable, as shown by the following experiment.

One mil of chenopodium, 29.57 mils of castor oil, and 237 mils of linseed decoction made by boiling 113 gm. of linseed in 296 mils of water, were thoroughly shaken together into a homogeneous emulsion. This amount was allowed for each of four hogs weighing 20.4 to 32.2 kgm. The emulsion was distributed evenly through a bran mash which all four hogs ate together. The animals had previously been fed a mash containing Epsom salt at the rate of 28.35 gm. for each animal, then starved for 24 hours before treatment. The fecal examination a few days after

the treatment showed that one hog was freed from ascarids, the three others still remaining infested.

It is evident that the administration of drugs by mixing them with the feed is a method which gives little promise of success, and the far greater efficacy achieved when the drug is given individually after a fast more than compensates for the extra labor involved.

In order to be certain that the comparative failure in this treatment resulted from the method of administration and not from an insufficient dose of chenopodium, the experiment was repeated, allowing 2.5 mils of oil to each of the three hogs remaining infested. This experiment resulted in a total failure, all the hogs remaining infested after the experiment.

The above experiments were concerned solely with the action of chenopodium against ascarids, no attention being paid to its action on other intestinal parasites. In order to test its action on nodular worms (*Oesophagostomum dentatum*) three doses of oil of chenopodium were given to one hog at intervals of 10 days, two fecal examinations being made after each dose. The chenopodium was given with 3.7 mils of areca nut after preliminary starvation, and, so far as could be judged from the fecal examinations, there was no diminution in the number of nodular worms. On account of their location in the cecum and colon, where they are protected by a large mass of fecal material, it is not surprising that a vermifuge given by the mouth has little or no effect upon nodular worms. The extensive colon of the pig is rarely, if ever, approximately empty, even after prolonged starvation. Probably the best way to reach worms in this location is by the use of diluted anthelmintics in the form of enemata, as suggested by Railliet (*1915*).

As *Chenopodium anthelminticum* grows abundantly in the vicinity of Washington, D. C., a test was made to determine the possibilities of feeding the entire plants to the hogs. The test was made when the plants were in full seed and therefore contained the maximum amount of oil. A large armful of chenopodium plants was placed daily in a hogpen containing three hogs. The amount given represented about all the hogs would clean up in a day, and the experiment continued for 19 days. Although the hogs received their usual daily ration, they ate the chenopodium plants readily. At the end of 19 days one hog was apparently free from ascarids (*Ascaris suum*).

While this treatment has the obvious disadvantage of inaccuracy in dosage and is applicable only in those regions where the chenopodium plant grows abundantly, it has the great advantage of costing nothing but the labor of gathering the weeds. At least it would seem worthy of further trial.

A test of the dried seeds of chenopodium was made in the following experiment: Each of three infested hogs was given daily 3 gm. of chenopodium seed and 3 gm. of areca nut mixed with the feed. The

treatment was continued for 11 days, at which time each hog had received 33 gm. of chenopodium seed, or the equivalent of about 1 mil of the oil. One of the three hogs was apparently freed from ascarids.

The treatment, besides being of uncertain efficacy, cost more than a single treatment with chenopodium oil, since a large amount of both chenopodium seed and areca nut must be consumed. Its efficacy would doubtless be increased if given after a period of starvation, but this is obviously impossible when the treatment must be continued for several days.

It appears from the above experiments that oil of chenopodium is an excellent anthelmintic for ascarids in hogs when given in suitable dosage after a preliminary period of starvation. The best purgative with which to combine it is castor oil. This has the advantage of relative cheapness, certainty of its action, and of being easily miscible with oil of chenopodium. The chenopodium may be given first and followed immediately by castor oil, or the two may be given together. The efficacy of oil of chenopodium is greatly decreased or entirely lost if the drug is mixed with the feed and several animals allowed to dose themselves while eating together. Chenopodium seed and areca nut have but little efficacy when given daily, mixed with the feed, and the expense of this treatment, on account of the number of doses required, is actually greater than a single dose of the relatively high-priced oil of chenopodium. Chenopodium plants seem to have some value as an anthelmintic and, when these are available, the cost of anthelmintic treatment is greatly reduced. However, further experimentation is desirable along this line.

The following experiments with chenopodium, in which the hogs were subsequently killed and all worms counted, confirmed the writers' opinion regarding the efficacy of oil of chenopodium when given under suitable conditions:

Two pigs weighing 8.16 and 14.06 kgm., respectively, were given 1 mil of oil of chenopodium, while a third, weighing 9.98 kgm., was given 2 mils. The drug was followed by 59 mils of castor oil in the case of one pig and given in 59 mils of castor oil in the case of the second pig. Eleven ascarids and six nodular worms were passed by the pigs. Post-mortem examination showed that all animals were freed from ascarids (*Ascaris suum*), while numerous nodular worms (*Oesophagostomum dentatum*) were found in each animal. The experiment adds further proof of the efficacy of oil of chenopodium as an ascaricide, and demonstrates that it also has some efficacy for nodular worms, although probably only a few were removed. As already stated, these worms are so protected by the large amount of fecal material in which they lie that it is unlikely that any anthelmintic given by the mouth will have much effect upon them.

As already noted in the previous experiments, oil of chenopodium, when mixed with the feed of a number of hogs feeding together, had shown little or no efficacy as an anthelmintic. Since, however, in these

experiments the pigs were not killed and the results were based solely on the fecal examinations, it was decided to repeat the experiment, killing the pigs and counting the worms and thus determining definitely whether this method could be recommended.

A sow which had been starved for 24 hours was fed a mash of corn meal and bran into which had been stirred an emulsion consisting of 3.5 mils of oil of chenopodium, 60 mils of castor oil, and 473 mils (1 pint) of milk. Following the treatment the sow passed 19 stomach worms (*Arduenna strongylina*) and 13 nodular worms (*Oesophagostomum dentatum*). A post-mortem examination revealed the presence of 19 ascarids (*Ascaris suum*), 13 stomach worms (*A. strongylina*), and numerous nodular worms (*O. dentatum*). The treatment proved a complete failure in eliminating ascarids, thus confirming the opinion already expressed regarding the inefficacy of chenopodium when given mixed with the feed. The treatment removed over half the stomach worms present and demonstrates the possibilities of the drug for this species of parasite. Although it also had some efficacy against nodular worms, as usual the percentage removed was very small.

In cooperation with Dr. Ernest, of the Tuberculosis Eradication Division, of the Bureau of Animal Industry, the junior writer was recently enabled to make a practical test of the chenopodium treatment of swine on a large scale. The test was carried out at the Green Berry Point Farm of the United States Naval Academy, where about 176 swine ranging from young pigs weighing 15.88 kgm. or less to large boars and brood sows, were given the treatment.

The pigs were starved for 24 hours before treatment and confined in relatively small pens so that they could be easily caught. The brood sows and boars were kept separate. The pigs were caught in the small pen and lifted one at a time over the fence into a larger inclosure, where they were treated. Four laborers were employed in this work, two capturing the pigs and two holding the animals while they were dosed. Restraint was made fairly easy by backing the pig into a corner of the inclosure. While one attendant held the pig in this position the other kept the animal's jaws apart, using two loops made of harness straps; one loop, passing under the upper jaw, was pulled upward while the other loop, passing over the under jaw, was pulled downward. The head was kept tilted upward and the pressure on the straps, besides keeping the mouth wide open, served to hold down the tongue and prevent the hog from shaking its head.

A table to hold the bottles of chenopodium and castor oil and the measuring glasses was placed conveniently near. Four mils of oil of chenopodium and 1 ounce (29.57 mils) of castor oil was allowed each hog under 100 pounds (45.36 kgm.), while those over that weight were given double the dose. No attempt was made to weigh the animals, but the dose for each hog was decided upon as it was brought up for treatment. The

two oils were measured separately, and were then poured together into a large iron kitchen spoon, which was placed as far back as possible in the animal's mouth. Subsequently, however, the iron spoon was dispensed with, the oils being poured directly into the hog's mouth, care being taken to hold the graduate glasses out of reach of the hog's teeth.

When the medicine had been given the pressure on the straps was relaxed to enable the hog to swallow, the head still being held high. If the hog refused to swallow, it could always be induced to do so by plugging the nostrils with the finger tips. This forces the animal to breath through its mouth, and to do so it must first swallow.

We found this method fairly rapid for hogs weighing not over 100 pounds (45.36 kgm.), 65 animals being dosed in one hour. It took eight hours to dose the entire herd of 176 hogs, the labor of handling the heavy brood sows making the operation much slower than when pigs of medium size were being treated. In treating the brood sows it was necessary to throw them on their backs and to hold them in this position while dosing. As this caused considerable excitement to the sows, those which from their appearance were soon to farrow were left untreated.

Only one of the 176 hogs treated was injured. This animal, which was accidentally dropped, injuring the spinal cord, was killed and examined for worms. One hundred ascarids (*Ascaris suum*) were taken from a piece of intestine not over 30 cm. long, and many more remained uncounted. Two days later the manager of the farm reported that a great many ascarids were seen among the feces on the place, and about two weeks later one of the pigs which was killed for food was found to be entirely free from ascarids. The treatment therefore appears to have been very successful. The treatment required 1.13 kgm. of oil of chenopodium and 7.5 liters of castor oil. With chenopodium at $11 per kilo ($5 a pound) and castor oil at 66 cents per liter, the treatment in this instance cost a trifle under 10 cents per hog, exclusive of the labor. Even at the present high price of drugs, the cost is trifling compared with the increased profit which may be expected to be derived from healthy animals.

For worms in sheep.—Four lambs weighing 16.6 to 26.1 kilos were dosed with oil of chenopodium at the rate of 0.2 mil per kilo, the dose which was found most effective for dogs. The medicine was given as a drench emulsified with 147.9 mils of milk. Following the treatment, one of the lambs contracted pneumonia, probably as the result of some of the drench's entering the lungs. The treatment succeeded in removing all the stomach worms from three lightly infested lambs, but failed completely in the case of one heavily infested lamb, suggesting that in this case the drench did not reach the fourth stomach directly, but was modified or absorbed in the rumen.

The treatment was fairly efficacious for hookworms (*Bunostomum trigonocephalum*), removing two-thirds of those present, in this respect

being considerably more efficacious than it was found to be against hookworms in dogs. It was inefficacious for other intestinal worms. On the whole, the use of chenopodium for stomach worms and hookworms in sheep seems to promise considerable success when properly administered, and is at least worthy of further trial.

For worms in poultry.—To test the efficacy of oil of chenopodium against worms in poultry, six chickens were dosed at a rate of about 0.4 mil per kilo. Each bird weighed 0.5 kgm. Each bird received 2 mils of castor oil followed at once by 0.2 mil of oil of chenopodium mixed with 2 mils of castor oil, the birds being kept without feed the previous day. The treatment was fairly satisfactory for *Ascaridia perspicillum*, removing 9 out of 13 worms. In this connection it will be recalled that the experiment in feeding tobacco stems to chickens was carried out with birds not infested with *A. perspicillum*, so that the writers have no data of their own to compare with the showing made by oil of chenopodium. As already stated, Herms and Beach (*1916*) found the treatment very efficacious for worms which were evidently *Ascaridia perspicillum*, to judge from their illustration of the parasites.

Chenopodium removed only 2 out of 349 cecum worms (*Heterakis papillosa*) and was entirely inefficacious against other nematodes. No tapeworms were passed in the feces, but 22 were found post-mortem, 2 of which were in the large intestine. Even though these 2 are considered as having been removed by the anthelmintic, the showing is not very creditable. It may be stated, however, that none of the substances tested by the writers for tapeworms on poultry have proved very satisfactory.

DISCUSSION OF RESULTS

In order to present the foregoing data in a condensed comprehensive summary, the various experiments have been tabulated by hosts (Tables I–V). These tables show, for each host, the efficacy of the different drugs tested against the more important parasites, as indicated by the percentage of worms removed compared with the total number present. Three columns of figures are given for each parasite, in order that the reader may see at a glance not only the percentage of efficacy but the data from which this percentage is derived, and thus be able to judge how conclusive or otherwise the figures presented may be. The tables also show the number of host animals used for each drug tested, and the size of the dose, usually based on the weight of the animal. A reference column gives the pagination of the experiments described in detail in the text. Where several experiments have been conducted in testing one drug, the results are combined in the tables into a single set of figures.

While it is realized that in most instances these data are insufficient to express results in percentages, and for this reason reference to the percentage of efficacy of a drug has been avoided in the text, it was thought that comprehensive tables like the following would prove convenient in summarizing the results and would at least serve to indicate what drugs offer promise of success. Too much emphasis, however, should not be placed on negative results based in most cases on insufficient data.

The tables do not include some of the earlier experiments carried out on hogs, in which the efficacy of the drug tested was determined solely by the results of fecal examination without killing and examining the animals post-mortem.

TABLE I.—*Percentage of efficacy of various anthelmintics for dogs*

Drug tested.	Reference to experiment.	Number of animals treated.	Dose.[a]	Ascarids.			Hookworms.			Whipworms.			Dipylidium.			Taenia.		
				Total number.	Number passed.	Percentage of efficacy.	Total number.	Number passed.	Percentage of efficacy.	Total number.	Number passed.	Percentage of efficacy.	Total number.	Number passed.	Percentage of efficacy.	Total number.	Number passed.	Percentage of efficacy.
	Page.																	
Calomel and castor oil	399	4	260 to 324 mgm.	19	1	5	......	......	......	......	......	......	......	......	......	......	......	......
Calomel (summary of several experiments).	......	5	194 to 324 mgm.	33	0	0	5	1	20	3	0	0	6	(*b*)	0	......	......	......
Epsom salt	400	3	4 gm. per 5 kilos	7	0	0	21	0	0	331	0	0	40	0	0	1	0	0
Castor oil (summary of 11 experiments).	399	50	3 to 40 mils	351	27	8	1,062	1	0.09	464	2	0.4	203	(*b*)	......	3	0	0
Chloroform and castor oil	403	5	0.2 mil per kilo	4	1	25	830	474	57	48	7	15	255	0	0	......	......	......
Iodoform [c]	405	4	0.2 gm.	(*d*)	......	......	(*d*)	......	......	(*d*)	......	......	(*d*)	......	......	(*d*)	......	......
Ether with castor oil	405	4	0.8 mil per kilo	23	8	35	503	0	0	......	......	......	......	......	......	1	(*b*)	100
Copper sulphate [e]	408	4	0.5 gm.	......	......	......	......	......	......	......	......	......	......	......	......	......	......	......
Coal-tar phenols	414	2	0.48 mil	5+	2	40	3	0	0	......	......	......	......	......	......	(*f*)	0	0
Oleoresin of aspidium (summary of 2 experiments).	414	6	1 to 3 mils	33	5	15	18	1	6	16	0	0	6	4	67	4	4	100
Areca nut and soluble cream of tartar.	418	3	2 gm. areca nut and 12 gm. cream of tartar.	67	4	6	0	0	0	0	0	0	......	......	......	......	......	......
Santonin [g] (summary of 3 experiments).	420	15	16 to 130 mgm.	96	23	24	35	0	0	87	6	7	5	0	0	2	0	0
Thymol and calomel [h] (summary of 3 experiments).	423	9	298 to 1,752 mgm.	18	10	56	151	23	15	419	13	3	77	2	3	1	0	0
Turpentine and castor oil	426	4	2.5 to 7.7 mils	(*i*)	2	(*j*)	(*f*)	0	(*k*)	......	......	(*k*)	......	......	......	......	......	......
Ficus laurifolia [l] and castor oil	427	3	11.7 to 47.3 mils	11	9	82	33	1	3	50	1	2	18	0	0	......	......	......
Spigelia and calomel	428	3	1.5 to 6 mils	18	1	6	205	6	3	29	2	7	1	(*b*)	0	......	......	......
Chenopodium and castor oil (summary of 6 experiments).	430	34	0.1 to 0.3 mil per kilo	200	194	97	370	119	32	398	46	12	1,794	1	0.05	2	0	0
Pelletierine tannate and castor oil.	417	3	305 to 363 mgm.	3	0	0	9	0	0	5	0	0	1	0	0	(*i*)	(*b*)	100(?)

[a] The dose indicated is for the anthelmintic, not for the laxative with which it is combined.
[b] Segments.
[c] No parasites passed. Only two dogs killed, which were found infested with ascarids, whipworms, Taenia, and hookworms. Treatment proved entirely inefficacious.
[d] Present post-mortem.
[e] Prompt emesis; no anthelmintic action.
[f] Present, as determined by a microscopic examination of the feces.
[g] Showed 70 per cent efficacy when given in repeated doses and 100 per cent in individual cases.
[h] Showed 100 per cent efficacy against ascarids and 50 per cent against hookworms, in repeated doses.
[i] Unknown.
[j] Low efficacy for ascarids.
[k] Ineffective for hookworms and whipworms.
[l] Experiments with the liquid expressed from the latex of *Ficus laurifolia* showed that this substance had apparently no anthelmintic value (see page 427).

TABLE II.—*Percentage of efficacy of various anthelmintics for swine*

Drug tested.	Reference to experiment.	Number of animals treated.	Dose.[a]	Ascarids. Total number.	Ascarids. Number passed.	Ascarids. Percentage of efficacy.	Whipworms. Total number.	Whipworms. Number passed.	Whipworms. Percentage of efficacy.	Nodular worms. Total number.	Nodular worms. Number passed.	Nodular worms. Percentage of efficacy.	Stomach worms. Total number.	Stomach worms. Number passed.	Stomach worms. Percentage of efficacy.
	Page.														
Epsom salt in solution [b]	400	2	75.6 to 227 gm. in water.	....	....	....	....	....	....	....	....	....	....	....	....
Tartar emetic and castor oil.	401	2	260 mgm. in water.	29	5	17	9+	6	....	786	26	3	....	....	....
Chenopodium and castor oil.	436	3	1 to 2 mils	11	11	100	(c)	0	0	(d)	6	....	1	0	0
Emulsion of chenopodium and castor oil mixed in feed.	437	1	3.5 mils	19	0	0	0	0	0	(d)	13	....	32	19	59

[a] The dose indicated is for the anthelmintic, not for the laxative with which it is combined.
[b] Entirely ineffective. Pigs would not drink either the stronger or the weaker solution.
[c] Present.
[d] Numerous.

TABLE III.—*Percentage of efficacy of various anthelmintics for sheep*

Drug tested.	Reference to experiment.	Number of animals treated.	Dose.[a]	Stomach worms. Total number.	Stomach worms. Number passed.	Stomach worms. Percentage of efficacy.	Hookworms. Total number.	Hookworms. Number passed.	Hookworms. Percentage of efficacy.	Nodular worms. Total number.	Nodular worms. Number passed.	Nodular worms. Percentage of efficacy.
	Page.											
Chloroform and castor oil.	404	[b]2	5 to 10 mils	14	9	64.0	5	0	0	6	1	17
Copper sulphate:												
In capsule	406	2	0.5 gm	10,541	41	0.3	....	....	....	327	0	0
In drench	406	3	(50 mils of 1 per cent solution.)	726	674	93.0	....	....	....	341	2	0.6
Gasoline in milk. (Summary of 3 experiments.)	409	9	7.5 to 30 mils	359	125	35.0	273	15	5	351	57	16.0
Petroleum benzin in milk.	411	2	15 mils	8	7	88.0	30	22	73	133	0	0
Coal-tar phenols in milk.	413	[c]6	2.8 to 15 mils	....	....	....	....	....	....	....	....	....
Chenopodium	438	4	3.3 to 5.2 mils in 147.8 mils milk.	978	38	4	29	19	66	239	22	9

[a] The dose indicated is for the anthelmintic, not for the laxative with which it is combined.
[b] Both sheep died following the treatment.
[c] Two sheep died; the others passed no worms.

TABLE IV.—*Percentage of efficacy of various anthelmintics for poultry*

Drug tested.	Reference to experiment.	Number of animals treated.	Dose.[a]	*Ascaridia perspicillum.* Total number.	*Ascaridia perspicillum.* Number passed	*Ascaridia perspicillum.* Percentage of efficacy.	*Heterakis papillosa.* Total number.	*Heterakis papillosa.* Number passed.	*Heterakis papillosa.* Percentage of efficacy.	Tapeworms. Total number.	Tapeworms. Number passed.	Tapeworms. Percentage of efficacy.
	Page.											
Areca nut with olive oil	419	6	1 gm	18	0	0	389	20	5	132+	1	....
Turpentine and castor oil	425	6	2 mils	66	50	76	463	10	2	444	8	2
Tobacco stems and Epsom salt	428	6	454 gm. per 100 birds.	....	....	....	162	30	19	42	3	7
Chenopodium and castor oil	439	6	0.2 mil	13	9	69	349	2	0.6	22	2	9

[a] The dose indicated is for the anthelmintic, not for the laxative with which it is combined.

TABLE V.—*Percentage of efficacy of various anthelmintics for cats*

Drug tested.	Reference to experiment.	Number of animals treated.	Dose.[a]	Ascarids.			Taenia.			Hookworms.		
				Total number.	Number passed.	Percentage of efficacy.	Total number.	Number passed.	Percentage of efficacy.	Total number.	Number passed.	Percentage of efficacy.
	Page.											
Oleoresin of aspidium and calomel....	416	[b]6	0.8 mil........	22	1	5	4	3	75	1	0	0
Pelletierine tannate and castor oil[c] ..	417	2	64 mgm. per kilo.	....	....	....	....	....	....	....	....	....

[a] The dose indicated is for the anthelmintic, not for the laxative with which it is combined.
[b] Two cats died shortly after treatment. The third cat vomited one Taenia.
[c] Treatment was entirely ineffective. No tapeworms or nematodes removed.

CONCLUSIONS

Making due allowance for the paucity of data in regard to certain drugs, the writers consider that the following may be reasonably advanced as the result of their investigations.

Simple purgatives, calomel and castor oil, may have some slight value as anthelmintics, but it is hardly sufficient to justify their use for this purpose. Ascarids in dogs are sometimes removed by castor oil given as a preliminary purge, and this fact may prove of benefit in veterinary practice as a diagnostic measure when the more accurate method of microscopic fecal examination can not be carried out. However, castor oil failed to remove ascarids more frequently than it succeeded, and in no case were all the ascarids removed from any one animal. As many of the experiments on dogs were preceded by a dose of castor oil, the writers have fairly extensive data on this subject.

The most reliable vermifuge for ascarids, whether in dogs or swine, is oil of chenopodium. This drug, which was tested out on 34 dogs in six experiments, showed an efficacy for the entire series of 97 per cent. It rarely fails to remove all the ascarids present in a dog if given at the rate of 0.2 mil per kilo, preceded by a dose of castor oil and the animal starved for 24 hours before treatment.

The chenopodium treatment is also very efficacious for ascarids in swine, and when properly administered may be expected to remove most, if not all, of the worms present. It would seem, however, that neither chenopodium nor any other drug tested will give satisfactory results if mixed with the daily ration and the animals allowed to dose themselves; it is best given to each pig individually in suitable dosage, preceded by a fast. While this method necessarily involves considerable labor when treating animals as unruly as swine, the labor can be reduced by sorting the hogs roughly into classes according to size and confining them in inclosures which will permit them to be caught with a minimum amount of struggling. The treatment has proved practical on a large

scale and the results, as far as they could be determined, were entirely satisfactory.

Oil of chenopodium appeared to be effective for stomach worms in sheep, although the data on this subject are not sufficient to warrant its recommendation. It is also of some efficacy for hookworms in sheep and in dogs, though in the latter case chloroform was found more reliable.

Other remedies which seem to have more or less merit as anthelmintics against ascarids are the latex of *Ficus laurifolia*, santonin in repeated doses, and thymol. Although thymol in repeated doses is fairly efficacious against hookworms, it was inferior to chloroform for this purpose, causing more distress. An excellent preparation for mixed infestation in dogs consists of equal parts of oil of chenopodium and chloroform, given at the rate of 0.2 mil per kilo, combined with 30 mils of castor oil. This preparation may be expected to remove all the ascarids present, a large proportion of hookworms, and possibly a certain percentage of whipworms. This latter parasite seems to be very difficult to eliminate, and nothing tried by the writers proved very efficacious, almost any anthelmintic occasionally proving successful. This experience may perhaps be explained by an intermittent peristalsis of the cecum, which occasionally allows the anthelmintic to enter, but which usually excludes it. Although chloroform was fairly successful in removing stomach worms from sheep, both animals upon which it was tried subsequently died from its effects, and it would seem to be too dangerous for use on sheep.

In the case of stomach worms in sheep, copper sulphate was found to be the most satisfactory remedy, the experiments confirming the findings of Hutcheon. A simple apparatus (fig. 1) devised by the senior writer reduces the labor involved in drenching a flock of sheep and insures accurate dosage. Petroleum benzin also proved satisfactory and was more efficacious for hookworms than copper sulphate. However, it is much more expensive than copper-sulphate solution, must be given three times, and in a vehicle like milk, which adds greatly to the expense. The fact that petroleum benzin (refined gasoline) proved efficacious, while commercial gasoline was considerably less so, is perhaps related to the differences in specific gravity and consequent volatility of the refined product compared with the commercial product.

Among anthelmintics intended for use against tapeworms, male-fern proved efficacious when tested on dogs. In the case of cats it removed all tapeworms from 75 per cent of the animals tested, though it proved fatal to two out of six animals which were somewhat enfeebled from disease. Apparently it is more toxic to cats than dogs and should be prescribed with caution and only given to healthy subjects. So far as can be judged from a single experiment with dogs, there seems to be no danger in combining male-fern with castor oil, as is done in the so-called Hermann's mixture. In fact, the writers are inclined to agree with Seifert

(*1908*) that the administration of castor oil after male-fern will avoid the toxic effects of the latter by causing its rapid and thorough elimination, and that for this purpose no other purgative is quite so effective. This subject, however, should receive more study before conclusions are drawn.

Pelletierine tannate was a failure in the one experiment in which it was tested on cats, but was efficacious on dogs. No remedy was efficacious against tapeworms in poultry. Of the four drugs tested, chenopodium gave the best results for this purpose, but its efficacy for tapeworms is very slight.

Turpentine proved the most efficacious of the remedies tested on poultry for the removal of *Ascaridia perspicillum*, while chenopodium was nearly as good. When tested on dogs and pigs, turpentine was not very efficacious; and, as it caused grave symptoms of nephritis in pigs and caused the death of some of the experiment dogs, its use upon these animals is inadvisable.

The treatment with chopped tobacco stems recommended by Herms and Beach for ascarids in poultry proved fairly efficacious for *Heterakis papillosa* and would presumably be at least as efficacious for *Ascaridia perspicillum*, since this latter worm is more easily reached by anthelmintics than is *H. papillosa*.

There are a large number of drugs showing a greater or less degree of efficacy for the various intestinal parasites of domestic animals. Usually their action is selective—that is, they show a pronounced efficacy for certain species of intestinal worms, while they are decidedly less efficacious or entirely inefficacious against other intestinal parasites. If we consider the ideal anthelmintic one which will remove all worms of a given class or species, and do this every time in a single dose, we find that very few drugs approach this ideal.

Among the drugs which have given the best results under experimental conditions for the purposes intended and concerning which the writers have sufficient data to warrant positive conclusions may be mentioned the following:

(1) Copper sulphate in drench for stomach worms in sheep.
(2) Oil of chenopodium for ascarids in pigs and dogs.
(3) Oleoresin of male-fern for tapeworms in dogs.
(4) Turpentine for *Ascaridia perspicillum* in fowls.
(5) Chopped tobacco stems for *Heterakis papillosa* in fowls.

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TOBACCO WILDFIRE[1]

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INTRODUCTION

During the past season (1917) a leaf disease of tobacco (*Nicotiana tabacum*) has been the cause of much concern to tobacco growers because of its destructiveness. Attention was first directed to it during June when tobacco was being transplanted. Subsequently during the entire growing season numerous complaints of this disease were received by members of the staff of the North Carolina Agricultural Experiment Station and of the State Department of Agriculture. Because of the severity of the epidemic and the insistence by growers that this disease was manifestly different from any they had ever seen, an investigation was begun.

A preliminary survey of the literature on diseases of tobacco revealed the fact that this disease was clearly unlike any which had previously been described. Furthermore, the apparently water-soaked margin of the diseased areas, the tissues of which, upon microscopic examination, were found to be teeming with bacteria, suggested that the disease was probably of bacterial origin. Accordingly, attention in the first studies was centered upon the etiology of the disorder. A brief statement (7),[2] concerning this work, in which attention was directed to the presence of the disease, was duly prepared.

It was also pointed out that the causal organism was undescribed, and the name "*Bacterium tabacum* Wolf and Foster" was suggested. Promise was made in that report of a description of the morphological and cultural studies upon *Bact. tabacum*. Besides making this description, it is the present purpose to adequately describe the disease and to indicate our present knowledge of its economic importance, distribution, and dissemination.

HISTORY AND DISTRIBUTION OF THE DISEASE

While the disease was first definitely recognized in June, 1917, near Wendell, N. C., it is impossible to determine with certainty for how many seasons prior to the present one the disease has existed. It is quite probable, to judge from the testimony of several reliable informants, that the disease caused the loss of practically the entire crop in one field, near Wendell, in 1916. Mr. E. G. Moss, Assistant Director, in Charge of the Granville Branch Tobacco Station, Oxford, N. C., is convinced, as is also

[1] Approved for publication by B. W. Kilgore, Director, North Carolina Agricultural Experiment Station.
[2] Reference is made by number (italic) to "Literature cited," p. 458.

the senior writer, that the malady was observed by him during 1916 at Creedmoor, N. C. To judge from the additional fact that the disease has been collected during the past season in 19 counties within North Carolina (Surry, Stokes, Forsythe, Guilford, Rockingham, Caswell, Alamance, Orange, Person, Durham, Chatham, Moore, Hoke, Wake, Johnston, Franklin, Granville, Vance, and Warren) and in 3 within Virginia (Pittsylvania, Halifax, and Mecklenburg) it is highly probable that the disease existed prior to the present year. Whether or not this disease occurs in other of the States in which tobacco is grown is not known, except in the case of Wisconsin. A letter from Prof. James Johnson, Department of Horticulture, University of Wisconsin, who has had occasion to observe the disease in North Carolina, states that a similar bacterial spot, although not nearly so destructive as in North Carolina, has been observed by him in Wisconsin.

ECONOMIC IMPORTANCE

The disease is universally conceded by growers of tobacco to be the most destructive one which attacks this crop. Losses, ranging from those which were inappreciable to those in which almost the entire crop was destroyed, were sustained in every locality where the disease was present. In some instances fields upon the same farm were observed to be badly affected, while others had little or none of the disease. In some sections, too, the disease occurred upon every farm within a radius of several miles, while in others it was present only in an occasional field. It is not possible, therefore, because the disease was not uniformly destructive over the entire area in which it is known to occur and because time has not been afforded to make a careful survey, to obtain a reasonable estimate of the damage wrought. Some idea of the losses, however, can be gained from statements taken from reports kindly furnished by a number of growers, who compared their leaf-tobacco sales with neighbors, whose crops were free from wildfire or were at most only slightly affected. Some of these reported losses averaging $100 per acre for their entire crop. One correspondent estimated his total loss at $5,000 and said that hundreds of farmers in his section suffered an equal acreage loss.

APPEARANCE OF THE DISEASE

The disease was first noted early in June during a period of rainy weather accompanied by nights which were so cold as to retard the growth of tobacco. The affected plants in many fields perished, necessitating replanting a second or a third time. A period of relatively dry, warm weather of about a month's duration followed, during which time, the crop made an extremely rapid growth, as shown by the fact that the plants were sufficiently mature to be topped. At this stage of their development another rainy season of about a week's duration occurred and was followed by another epidemic of the disease. The disease

appeared so quickly, spread so rapidly, and affected the leaves so seriously that it was commonly given the appropriate designation "wildfire."

The foliage alone seems to be subject to attack. The first evidence of disease is the appearance of circular, chlorotic areas varying from 0.5 to 1 cm. in diameter. Within 24 hours after this chlorosis is first noted minute brown areas will have formed at the centers of the spots (Pl. 15, A). Within another day these spots will have enlarged greatly Pl. 15, B), and a border of water-soaked appearance marks the margin of the necrotic tissues. Within a few more days the diseased areas are 2 to 3 cm. in diameter and are often strikingly concentric with shades of tan to dark brown, the centers being lightest in color (Pl. 16, A). Such spots have a broad translucent border, which is in turn surrounded by a chlorotic halo that pales out into adjacent tissues (Pl. 15, B; 16, A). When the spots are numerous, they fuse, causing large, irregular areas of leaf tissue to become dry. These dead areas remain intact in case there is no precipitation. When dewy nights and intermittent showers occur, however, the dead areas rot out so that the leaves present a ragged appearance (Pl. 16, C) which is especially manifest when large numbers of infections occur upon a single leaf.

Not uncommonly the leaves on one side of the plant are more seriously diseased than those on the opposite side and there may even be a unilateral destruction of these leaves resulting in distortion, as shown in Plate 16, B. The vascular tissues seem not to be invaded, but the organism confines its attack to parenchymatous tissues.

Two other leafspot diseases of tobacco, frogeye and speck, are present within North Carolina, from both of which wildfire is easily distinguishable. Frogeye appears as circular, brown spots, with a darker border and with grayish centers. Upon this gray center may be seen the fructifications of *Cercospora nicotianae* E. and E., or other fungi associated with the disease. No chlorosis accompanies these spots. Speck, which results from a deficiency of potash, appears as tan-colored, irregular areas which are first present at a distance from the principal veins. When this disease is accompanied by chlorosis, there is no definite halo around the lesions. In the case of neither of these diseases is the margin of the affected areas water-soaked in appearance, and in neither of them do the affected tissues disintegrate and fall out.

ISOLATIONS AND INOCULATIONS

On June 13, fresh material of tobacco wildfire was collected and isolations were made by planting on poured plates of nutrient agar fragments of tissue from the margin of affected areas. Contamination was avoided by washing the leaves prior to making the planting in mercuric chlorid and then rinsing them in sterile water. Several types of colonies developed along the margins of these plantings, the most common of which was *Bact. tabacum*, which appeared as glistening, grayish white

colonies. It was possible, in some cases, to make transfers directly from these colonies to tubes of agar and secure pure cultures. In others, dilution poured plates were first made, and the organism was transferred from certain of the colonies which developed to tubes of agar. Diseased material was collected several times subsequently, and numerous specimens were received by mail, so that opportunity was afforded during the season to isolate the organism from several sources.

Some preliminary inoculations were made on June 13 in which the inoculum consisted of macerated, diseased leaves upon which a quantity of water was poured. About 100 young tobacco plants growing in a flat in the greenhouse were then sprinkled with this water. Four days later infections were evident by the appearance of numerous yellow spots with pin-point-like centers.

On the evening of June 28 two potted tobacco plants, about 18 inches in height, were inoculated with pure cultures of *Bact. tabacum*. A watery suspension from agar cultures was sprinkled upon these plants, after which they remained covered with a bell jar for 36 hours. On the morning of July 2 numerous chlorotic areas had formed, which by July 5 had changed to large dry spots, typical of wildfire. No difficulty was experienced in reisolating the organism from these lesions.

On July 10, 16 plants which had been transplanted in the field on June 14 were inoculated by sprinkling them with suspensions made from bouillon cultures. These plants were not seen again until July 15, when large brown areas had formed abundantly, whereas adjacent uninoculated plants remained healthy.

Another series of inoculations, involving 18 plants growing in pots placed outside of the greenhouse, was made on July 25. In this case the leaves were immersed in a bacterial suspension. Seventy-two hours later the first evidence of infection was observed. Here again the organism was recovered from mature lesions.

Another set of inoculations, involving 12 potted tobacco plants, was made with what proved to be *Bact. tabacum* isolated from spots on cowpeas (*Vigna sinensis*) which had been planted between the hills in a badly diseased tobacco field. The spots on cowpeas, from which these isolations were made, were very similar in appearance to 3- or 4-day-old lesions on tobacco. Eight tobacco plants were inoculated on July 27 and four on August 2. Inoculation was effected by sprinkling the plants with a bacterial suspension. By August 1 in the first case and August 9 in the second there was no doubt that the diseased areas, which had formed upon all of the inoculated plants, were typical of wildfire. The organism was reisolated from these spots and, together with transfers from the original cultures from cowpeas, was used in inoculating cowpeas. Here again the same method as before was used in making inoculations. Only a few spots developed upon the several plants employed in two sets of inoculations. These spots were similar to those on cowpeas growing

in the field of diseased tobacco. Microscopic examination, furthermore, showed that the dead tissues were filled with bacterial organisms.

The lesions, both naturally and artificially produced, are believed to have originated around punctures made by leaf-hoppers, which were abundantly present on these plants throughout the season. The wildfire organism is capable of multiplying within the cells weakened as a result of the withdrawal of their contents by the feeding of these insects, but is not able to parasitize normal cells. Drops of moisture laden with bacteria certainly dripped from the diseased tobacco plants to the cowpeas beneath them, and could thus have supplied the inoculum which caused the cowpea foliage to become spotted. This explanation is supported by the observation that the lesions on cowpeas did not increase in size beyond pinpoint-like dead areas, indicating that *Bact. tabacum* can not adapt itself to invade healthy tissues, and by the further fact that no new spots developed subsequently on the naturally and artificially inoculated plants. Furthermore, spots never developed on cowpeas growing at a distance from diseased tobacco plants—that is, where they could not be infected through the agency of water dripping from diseased tobacco plants. *Bact. tabacum*, therefore, is not parasitic upon cowpeas, and its chance occurrence upon this crop indicates that conclusions as to the pathogenicity of bacteria when judged from inoculation experiments in which the inoculum is introduced through wounds are not entirely convincing. In view, therefore, of the fact that tobacco is not grown in the vicinity of West Raleigh, where the inoculation experiments were conducted, and that all of the uninoculated plants within the greenhouse grew to maturity without any manifestation of wildfire, there is no doubt that all of the infections which were secured resulted from inoculations with the organism in hand. When judged by the readiness with which infection occurs, *Bact. tabacum* is to be regarded as a very vigorous pathogene.

Aside from the inoculations upon cowpeas, only two other host species, bell peppers (*Capsicum annuum*) and Jimson weed (*Datura tatula*), were employed, with negative results.

PATHOLOGICAL ANATOMY

Affected tissues were fixed in 95 per cent alcohol, embedded in paraffin, sectioned, and stained with carbol-fuchsin. The presence of a crystalline substance whose nature is described in a recent paper by Ridgway (*3*) interfered seriously with the cutting of suitable sections. In tissues in which the cells had not yet become dry and collapsed, bacteria are abundantly present within the intercellular spaces (fig. 1). In mature lesions, however, they occur also within the cells. The contents of such cells appear to have been completely destroyed, whereas the walls have undergone little disintegration. The complete disintegration of diseased tissues, which occurs in the presence of excessive moisture, results, it

is believed, from the activity of other species of bacteria which enter the cells following invasion by *Bact. tabacum.* These species appear always to be present in old lesions as judged by the isolation studies.

DESCRIPTION OF BACTERIUM TABACUM

Bacterium tabacum, emend.

The primary cause of tobacco wildfire is a grayish white, rod-shaped organism with rounded ends. It is motile by means of a single polar

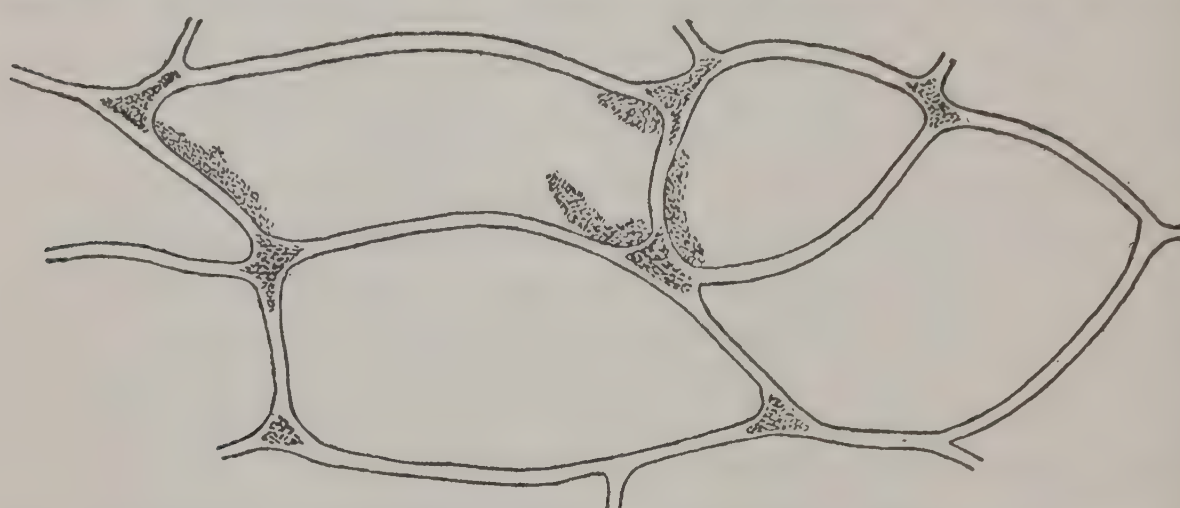

FIG. 1.—Parenchyma cells from the margin of a lesion showing *Bacterium tabacum* in the intercellular spaces and within the cells.

flagellum which is about twice as long as the body of the bacterium (fig. 2, *a*). Motility can be observed when fresh material is examined in a drop of water or when preparations are made from 18-hour-old bouillon or agar cultures. Flagella are easily demonstrated when the organism from such cultures is subjected to the staining method outlined by Morrey (*2*). The bacterium usually occurs singly within the host tissues, but in culture chains of as many as five elements have been noted (fig. 2, *b*). The limits of size vary from 2.4 to 5 by 0.9 to 1.5 μ, the most common size being 3.3 by 1.2 μ. No involution forms have been observed; neither have endospores been demonstrated.

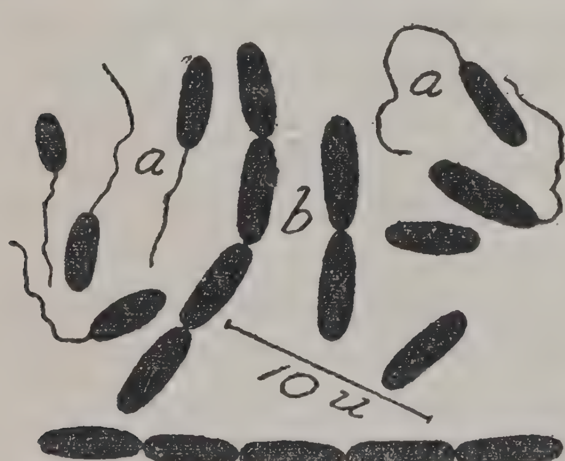

FIG. 2.—*a*, Flagella of *Bacterium tabacum* stained by Morrey's method; *b*, *Bact. tabacum* from bouillon stained with carbol-fuchsin, showing arrangement of the elements.

The organism stains readily with aqueous- and carbol-fuchsin, anilin gentian-violet, and methylene blue. It is Gram-negative, however, and is not acid-fast. Neither is a capsule demonstrable by the methods of Räbiger or Welch.

The organism has been cultured mainly upon glycerin agar and potato agar. Colonies appear in these media on the second day in poured plates

kept at a temperature of 20° to 25° C. By the fourth day the surface colonies have attained a diameter of 2 to 3 mm. They are grayish white in color, circular in outline, are appreciably raised, and have a smooth margin and a smooth, wet-shining surface. Buried colonies are biconvex.

In stroke cultures a filiform growth which widens at the base of the slant is formed. Growth is moderate and does not give rise to the production of odor. In stab cultures growth is best at the surface of the agar, and the line of puncture is filiform. In stab cultures on nutrient gelatin a filiform growth also appears along the line of puncture, with the greatest growth at the surface of the medium. No evidence of liquefaction occurs until the tenth day, when it becomes crateriform, and is complete within 30 days.

Growth on potato cylinders is nontypical in appearance, and there is no evidence of diastatic activity.

With a 2 per cent solution of Difco peptone as a basal solution, five solutions were prepared by adding 1 per cent of one of the following carbon compounds: Dextrose, saccharose, lactose, glycerin, and dextrin. No gas formed in fermentation tubes containing any of these media. A vigorous growth with strong clouding and a surface pellicle occurred in all in the open arm. In the presence of dextrose and saccharose a distinctly visible clouding gradually extended upward into the closed arm, while in the case of the other carbon compounds the closed arms remained clear. The organism is therefore regarded as aerobic in general.

Acid formation in stab cultures on litmus-glycerin, litmus-dextrose, litmus-lactose, and litmus-saccharose agar begins within four to six days, but no gas formation occurs on any of these media.

Growth on litmus milk presents a very characteristic appearance. During the first three days following inoculation there is a deepening of the blue color. Two days later coloration begins to become stratiform, and by the seventh or eighth day four distinct layers are evident. The upper one is between plumbeus and violaceous in color (*5*), the next is lilacinus, the third, violaceous, and the lowermost approximates cæsius. These colors lose their intensity after a few days and become more or less blended, and by the tenth day there is evident reduction of the litmus and precipitation of the casein. Reduction proceeds rather slowly and is complete by the twenty-fourth day.

There is no reduction of nitrates in nitrate-peptone broth, although a conspicuous clouding occurs. Furthermore, no gas is formed; and the tests for ammonia, indol, and skatol were negative.

The thermal death point of this organism, as determined by exposing newly inoculated tubes of bouillon in the customary manner, was found to be about 65° C. It is manifestly quite sensitive to desiccation, since no growth appeared after six days when bouillon cultures were placed on sterile slides in sterile petri dishes.

The group number of *Bacterium tabacum* according to the numerical system of the Society of American Bacteriologists is 2½1.2222032.

DISSEMINATION OF WILDFIRE

The fact that the disease appeared in epidemic form twice in one season and that each epidemic followed a rainy period, with little or no new infection in the interim suggests that dissemination is primarily influenced by moisture. This is in accord with observations on the dissemination of certain other plant diseases of bacterial origin, as angular leafspot of cotton (*4*) and Citrus canker (*6*).

Very striking evidence was found in two instances that wind is also a potent factor in the spread of wildfire. In one instance a field was observed before and several days following a rain which was accompanied by a high wind. The disease had advanced in consequence over a distance involving 16 to 18 rows lying parallel to diseased tobacco. The disease terminated rather abruptly beyond this distance. In another locality no disease occurred, except in the case of a field of approximately an acre in area, the plants for which had been brought for a distance of several miles. Here, again, the disease spread, following the same storm into an adjacent field to the leeward, was most abundant near the field of diseased plants, and gradually became less in the direction away from the diseased field. The organism had very evidently been spread by wind-blown rain, a phenomenon in accord with observations by Faulwetter (*1*) upon the angular leafspot of cotton.

When the first epidemic was prevalent in the vicinity of Oxford, N. C., thrips were abundantly present upon tobacco and were popularly suspected of being responsible for the spread of the disease. Accordingly, diseased leaves bearing numbers of these insects were collected, and the thrips were liberated upon healthy plants in the greenhouse at West Raleigh. Careful watch was kept, but no evidence of wildfire developed upon any of the plants. In the same season only a few thrips were found in diseased fields near Wendell, N. C., during an entire afternoon's search. For these reasons it is improbable that thrips are to be regarded as agents of dissemination.

Following the first outbreak of wildfire, opportunity was afforded to make numerous observations upon the origin of the disease. In every instance where the disease occurred in the field it has been possible to find that plants in the seed beds or "plant beds" were also affected. It was adjudged, therefore, that the disease must have been introduced into the plant beds either through the use of infected seed or through the agency of fertilizers. One large seed farm upon which diseased plants occurred was visited in searching for wildfire lesions upon seed pods, and, furthermore, affected pods were carefully sought for in many other diseased fields with negative results.

Since the disease is already so widely spread, which suggests that it must have had some common agency of dissemination, and since tobacco stems were incorporated in certain fertilizers as the source of potash, inquiry was directed to determine the possibility of the introduction and

dissemination of wildfire by fertilizer materials. Attempts to isolate *Bact. tabacum* from diseased leaves which had passed through the curing process gave negative results in the case of three samples tested. In view of the fact that no growth occurred in bouillon cultures exposed to temperatures above 65° C. for 10 minutes, as has previously been reported, it is highly improbable that the organism could survive for several hours temperatures of 180° F. and above, as are maintained for several hours in the last part of the curing process. Furthermore, in the preparation of tobacco stems for incorporation with fertilizer materials they are subjected to a sufficient degree of heat to insure complete sterilization.

SUMMARY

(1) A leafspot disease of tobacco called "wildfire," which is more destructive than any other malady affecting this crop, has appeared within North Carolina and Virginia.

(2) It has been collected during the past season in 19 counties within North Carolina, 3 within Virginia, and occurs also in Wisconsin.

(3) Wildfire first attracted attention at time of transplanting tobacco and appeared again in epidemic form at time of topping the crop.

(4) The disease originated in the seed bed or plant bed, but only negative evidence had been secured that infection comes from the seed.

(5) The leaves alone are attacked, and the symptoms are entirely unlike those of other foliage disease of tobacco.

(6) The primary cause of wildfire has been found to be a wet-shining grayish white, 1-flagellate organism, which is herein described as *Bacterium tabacum.* Its period of incubation is about 72 hours, and large lesions are formed within a week.

(7) The disease is of the necrotic type, involving parenchyma tissues.

(8) Moisture is of prime importance in the spread of wildfire. When rains are accompanied by wind, dissemination is especially rapid.

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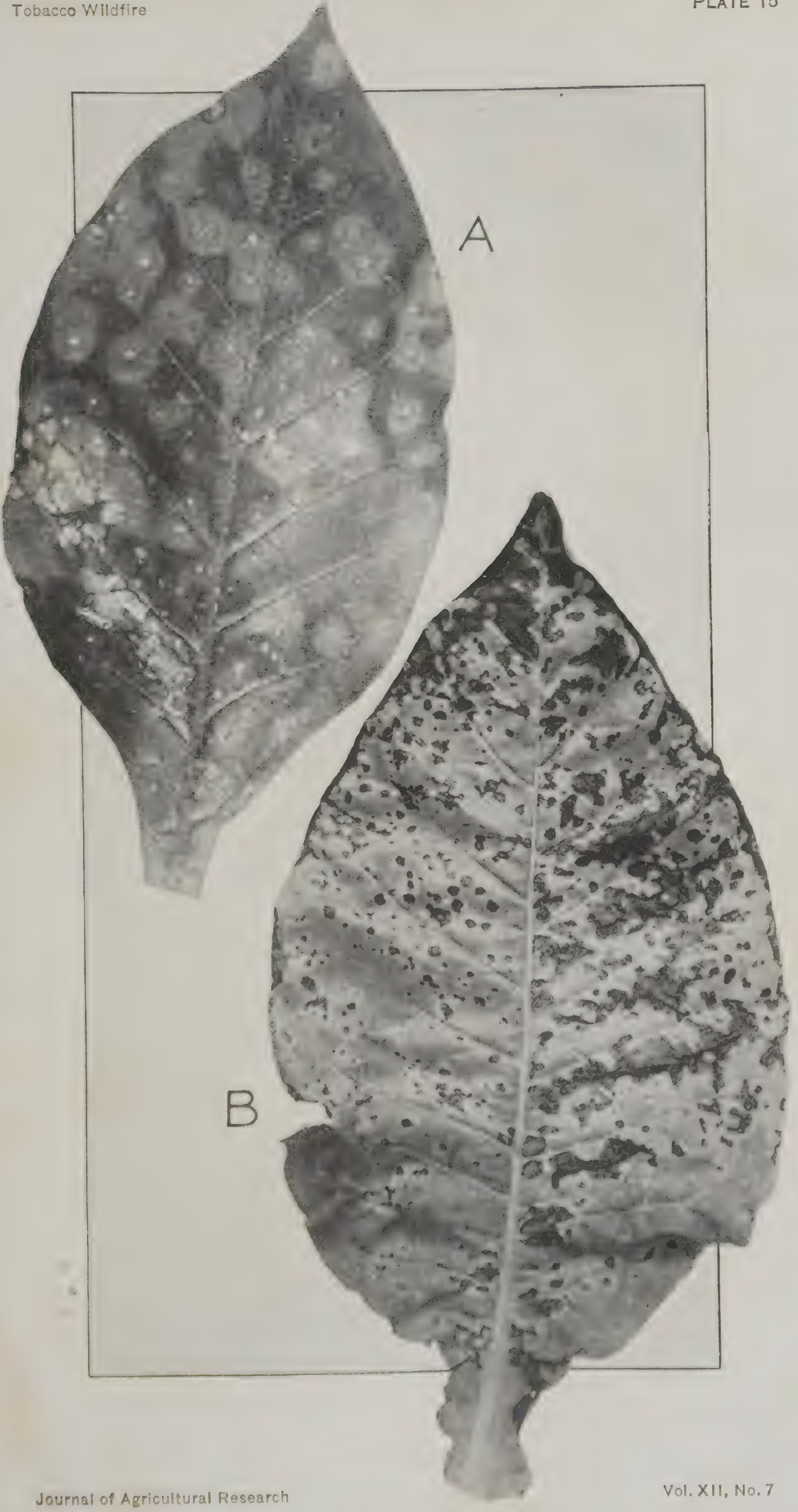
PLATE 15:

Bacterium tabacum:

A.—Tobacco leaf, four days after artificial inoculation, showing chlorosis and lesions.

B.—Natural infection with brown lesions bordered by tissues of a water-soaked appearance.


A
B


A
B
C

PLATE 16:

Bacterium tabacum:

A.—Natural infection. Lesions are large and concentrically zonate.

B.—Numerous confluent lesions on one side of the midrib have resulted in distortion of the leaf.

C.—Almost the entire leaf is involved and a portion of the rotted tissues have fallen out. Natural infection.

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GIPSY-MOTH LARVÆ AS AGENTS IN THE DISSEMINATION OF THE WHITE-PINE BLISTER-RUST[1]

By G. Flippo Gravatt, *Assistant Pathologist*, and G. B. Posey, *Scientific Assistant, Investigations in Forest Pathology, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

Very little has been done to correlate the widespread distribution of the white-pine blister-rust, caused by *Cronartium ribicola* Fischer, with factors governing the dissemination of the spores of the causal organism. The early occurrence of telia on leaves of currant and gooseberry plants (*Ribes* spp.) in localities distant from known infections on pines (*Pinus* spp.), together with the absence of definite knowledge of instances of overwintering on the former hosts, is suggestive of distant seasonal spread of the disease by æciospores from pines.

Larvæ of the gipsy moth (*Porthetria dispar* L.) feed on the Peridermium stage of *Cronartium ribicola* and carry thousands of æciospores on their bodies. As Collins[2] found that larvæ of the gipsy moth are blown as far as 20 miles, these insects are a potential agent in distant spread of the blister-rust. The gipsy moth is distributed over a large portion of the white-pine region of New England.

GIPSY-MOTH INFESTATION ON DISEASED PINE

In the fall of 1916 a stand of white pine covering an area of from 5 to 7 acres at Kittery Point, Maine, was found to be severely infected with *Cronartium ribicola*. This growth ranged from young seedlings to mature trees 80 feet tall and random ¼-acre plots in this area showed 65 to 100 per cent of the trees to be diseased. The number of infections on individual trees ranged from 1 to more than 300, and it was estimated that there were 75,000 to 100,000 separate infections in trees on this area.

In the infected plot gipsy-moth-egg clusters were found in varying abundance on limbs and stems of pines of all sizes, and were located from near the ground to the tops of the largest trees. In a number of cases egg clusters were present on the diseased bark, and in one instance four were located on a single canker.

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[1] The writers are indebted to Mr. A. F. Burgess, of the Office of Gipsy Moth and Brown-tail Moth Investigations, Bureau of Entomology, for helpful suggestions. Further work is being carried on in cooperation with the above office.

[2] Collins, C. W. Dispersion of gipsy moth larvæ by the wind. U. S. Dept. Agr. Bul. 273, 23 p., 7 pl. 1915. Bibliography, p. 22–23.

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On May 25, 1917, large numbers of gipsy-moth larvæ were found in and around ruptured blisters, and several days later some of the blisters where the presence of larvæ had been previously noted were empty, and spore production was apparently arrested. Subsequent observation on these blisters showed that no further spore production took place, while on the same cankers blisters which were artificially protected from larvæ continued to produce spores in abundance until June 25. To determine the rate of blister destruction on June 9 a number of larvæ were placed on a twig infection which had 38 sporulating pustules. Many of the larvæ crawled away or dropped off, but a sufficient number remained to destroy the fruiting layer in practically every blister by noon of the following day, with the result that no subsequent spore production took place.

Cessation of spore production in injured blisters was caused by the destruction of fruiting hyphæ. Usually the spores and hyphæ were eaten away first and then the larvæ very often ate through the base of the fruiting layer to a depth of several millimeters. Apparently, after blisters no longer furnished suitable food for the larvæ, they began feeding on the areas of the yellowish, discolored, infected bark outside the fruiting region and in some cases a large per cent of the outer bark of next year's sporulating zone was destroyed. Careful observations on many larva-infested cankers showed that spore production was prematurely arrested in 25 to 100 per cent of the pustules, the percentage usually averaging highest on small twigs.

SPORES ON LARVÆ

Larvæ working in blisters collected so many æciospores on their hairy bodies that they appeared nearly the color of spores in mass. On different dates larvæ were taken from blisters and placed in separate capsules, precautions being taken against including spores not on the bodies. These were taken into the temporary laboratory and spore counts made of the bodies and the alimentary tracts. Spores for counting were removed from larvæ by washing the bodies in series of water, and alcohol mounts on slides followed by final examinations to assure thoroughness in the method used. This procedure proved quite effective, and, where carried through 5 to 10 washings, practically all spores were removed from the outside of the bodies. Counts were made on the spores adhering to the inside of the capsule and added to the total found in the washings. After bodies of the larvæ had been thoroughly washed, they were dissected and counts made of spores in the alimentary tracts. On May 26, June 4, and June 11 fifteen small larvæ were collected. Spore counts on the bodies of these 15 gave a minimum of 1,120, a maximum of 28,320, and an average of 18,100. Counts of spores in the alimentary tract gave a minimum of 1,740, a maximum of 48,570, and an average of 26,022

To determine the approximate amount of spore material passed through the alimentary tract, 20 larvæ were placed on fresh cankers in a feeding tray. After they were settled and had fed normally for several hours, a sheet of paper was placed under the cankers for the collection of pellets. A total of 423 pellets were dropped within a period of 13 hours. Counts of the spores in these pellets gave from 3,960 to 12,450, with an average of 8,160, which is at the rate of 318,616 spores excreted per day per larva.

Germination tests made of the spores on the bodies of larvæ collected on cankers gave positive results, and approximately the same percentage of germination was observed as on spores taken directly from these cankers. Germination tests of spores in pellets have given very poor results; in only one case did several spores germinate. In many cases spores taken directly from these cankers also failed to germinate in laboratory tests.

WIND DISPERSION OF LARVÆ AS A FACTOR IN BLISTER-RUST SPREAD

At Kittery Point, Me., æciospores were produced from April 29 to July 1, with maximum spore production from May 10 to 25. Collins[1] gives the hatching period for gipsy moth larvæ in this section for 1912, 1913, and 1914 as May 1 to 23, April 29 to May 14, and May 11 to 28, respectively. The season of 1917 was approximately one week later than usual. The period of wind dispersion of larvæ is given as ranging from 18 to 30 days, starting one to two weeks after the first caterpillars hatched. Observations by the writers showed varying numbers of larvæ feeding on blisters from May 25 to June 25.

Collins[2] working with wind dispersion of larvæ of the gipsy moth over a series of several years showed that they were carried in wind currents to distances as great as 20 miles. The same author states that approximately 50 per cent of the larvæ caught at distances of 6 miles or less had fed previously.

The writers, using fly-paper traps, placed 10 to 30 feet from the nearest pine infection, and so arranged as to exclude larvæ that may have reached the trap by crawling, caught 75 small larvæ. Four of these caterpillars had, respectively, 35, 105, 185, and 2,180 æciospores on their bodies, which establishes the fact of local wind dispersion of æciospore-bearing gipsy-moth larvæ. That spores carried on bodies of larvæ may remain viable for a considerable length of time is borne out in viability tests under laboratory conditions, wherein æciospores germinated after remaining in vials for a period of two months.

Examination of wild and cultivated species of Ribes at various points throughout Kittery Township showed an abundance of gipsy-moth larvæ feeding on the foliage, and in many cases they were observed crawling on the under surfaces of leaves. Quite a number of the larva-

[1] COLLINS, C. W. 1915. Op. cit. [2] COLLINS, C. W. 1917. Op. cit.

infested plants showed areas producing uredospores, and in four instances the only leaves showing blister-rust infections were those which had been injured by insects.

Sixty larvæ collected on species of Ribes were examined for æciospores. Of these one larva collected on June 14 on the under surface of a wild gooseberry plant showed 280 æciospores and 520 uredospores on its body. The gooseberry plant was heavily infected with blister-rust, being located only 20 feet from pine infections. Germination tests of the spores from this larva gave two germinating æciospores and many germinating uredospores, thus bearing out the fact that gipsy-moth larvæ do carry viable spores to *Ribes* spp. and also showing the part which insects may play in local distribution of the disease by uredospores.

PRACTICAL IMPORTANCE

The facts given in regard to the gipsy-moth larvæ show that these insects are certainly a factor in the spread of the blister-rust locally from pines to *Ribes* spp. Their habit of feeding and crawling over the lower leaf surface, where the stomata are located, gives the spores borne on their bodies a good opportunity for causing infection. The probability of the spread of blister-rust from pines to distant *Ribes* spp. is undoubted, since Collins' work shows that the gipsy-moth larvæ are blown by winds of varying intensity for distances of 20 miles. Though wind is considered to be the most important factor in æciospore dissemination, gipsy-moth larvæ undoubtedly play an important part. Other insects have been collected from infected pines with thousands of æciospores on their bodies, but these insects were not present in sufficient numbers to make them of importance in comparison with the number of gipsy-moth larvæ present.

SUMMARY

(1) The period of hatching and of wind dissemination of gipsy-moth larvæ came within the period of spore production of the blister-rust on pines.

(2) Larvæ fed abundantly on spores and injured the fruiting layer of the pustules so that further spore production was arrested.

(3) Larvæ from blister-rust cankers had thousands of viable spores on their bodies. A small percentage of the larvæ collected from fly paper and from species of Ribes near infected pines showed æciospores on their bodies.

(4) Gipsy-moth larvæ were found feeding on leaves of *Ribes* spp., and in some cases the only infected leaves on plants of this genus were those showing insect injury.

(5) The Bureau of Entomology has shown that these larvæ are blown by the wind up to a distance of 20 miles. Within this distance the larvæ are potential agents in the spread of the white-pine blister-rust (within the area infested by the gipsy moth).

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INFLUENCE OF CARBONATES OF MAGNESIUM AND CALCIUM ON BACTERIA OF CERTAIN WISCONSIN SOILS[1]

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THE PROBLEM

In the course of a study of the biology of certain acid soils it was found that magnesium carbonate causes a great increase in the reproduction of soil bacteria. Just what effect this great increase in number of bacteria has on the fertility of the soil is a problem beyond the scope of this paper. However, it is a well-established fact that the majority of the changes of soil constituents are brought about by microorganisms. These substances, which are constantly undergoing changes, are influenced by the number and kinds of bacteria.

The beneficial effect of lime, calcium carbonate, and magnesium carbonate on the growth of higher plants is generally preceded by an increase in the number of bacteria. Evidence is sufficient to warrant the conclusion that an increase in the number of soil microorganisms is usually reflected in a more rapid decomposition of organic matter and a greater liberation of the inorganic soil constituents, chiefly potassium and phosphorous.

Although many important data have been obtained in relation to the effect of liming on the bacteria in soil, there still remain problems which deserve careful investigation—for example, the amount and kind of lime and calcium and magnesium carbonates that can be applied most economically and yet give the best results.

The investigations of this paper were undertaken with the hope of throwing some light upon the problem of soil bacteria and their response to applications of magnesium carbonate, calcium carbonate, and limestone.

The effect of these substances on pure cultures of bacteria and on the ordinary soil flora was observed in—

(*a*) Acid Colby silt loam soil;
(*b*) Acid Plainfield sand;
(*c*) Neutral Miami silt loam.

[1] This paper is submitted in partial fulfillment of the requirements for the degree of doctor of philosophy in bacteriology in the Graduate School of the University of Wisconsin, July, 1917.

[2] The writer is indebted to Dr. E. B. Fred, of the Wisconsin Experiment Station, for supervision and suggestions in the experimental work and preparation of the manuscript.

REVIEW OF LITERATURE

A complete review of the literature, showing the effect of lime (CaO), calcium carbonate ($CaCO_3$), and magnesium carbonate ($MgCO_3$) on soil microorganisms, is not attempted in this paper. Only a brief résumé of certain of the more important papers is given.

Although this paper is primarily concerned with the relation of soil microorganisms to calcium and magnesium carbonates, it was deemed worth while to include a brief review of results obtained from the use of lime and limestone. Under ordinary soil conditions, calcium oxid, or lime, is soon converted into calcium carbonate. Because of the frequent occurrence of magnesium in limestone, this substance was included in the discussion.

NUMBER OF ORGANISMS

In 1901 Chester (*7*)[1] made plate counts of an acid soil which had received lime at the rate of 1,000 to 4,000 pounds per acre. In every case the lime increased the total number of bacteria; the greatest gain was noted where the largest amount of lime was applied.

Fischer (*18*) treated soil with lime and calcium carbonate at the rate of 0.1 to 3.0 per cent by weight of soil. The calcium carbonate and lime were added in gram-molecular equivalents. His results showed that 0.1 and 0.2 per cent of lime after three days gave an increase in the total number of bacteria. Treatments amounting to 0.5 per cent and more were harmful at first, but later gave an increase beyond that of the control. The data showed that a slight increase in the number of bacteria occurred when calcium carbonate was added to the soil.

Several years later, Hutchinson (*23*) made somewhat similar experiments, using lime and calcium carbonate. He also found that lime at first exerted a depressing effect but later gave rise to an increase in the number of bacteria. He concluded that the reduction in the number of bacteria immediately after treatment with lime was due to the caustic effect of the oxid, since no injury was noted after the oxid changed to carbonate. He believed the benefit from liming was due in part to the gain in soluble organic matter, to the improvement in the physical condition, and to the correction of the acid reaction of a soil, all of which tended to bring about a better environment for the development of bacteria. However, this investigator held that these changes brought about by the action of liming did not seem sufficient to account for the enormous increase in plant growth. He assumed, therefore, that the action of lime was much the same as an antiseptic—that is, it caused a partial sterilization.

In a later publication, Hutchinson and MacLennan (*24*) reported the results of experiments with lime and calcium carbonate on five different soils. The range of reaction in these soils varied from neutral to strongly

[1] Reference is made by number (italic) to "Literature cited," pp. 500–504.

acid. Lime was added in amounts varying from 0.1 to 1.0 per cent, while calcium carbonate was added in amounts of 1.0 per cent only. Besides the total number of bacteria, these investigators determined the nitrate and ammonia nitrogen of the soils subjected to this treatment. The results of the experiments showed that in all of the soils, liming increased the number of bacteria. At first the heavier applications of lime retarded and later stimulated the reproduction of microorganisms. The acid soil required more lime to retard the growth of bacteria than the nonacid soils. As a result of liming, ammonification and nitrification in all of the soils was increased. The authors concluded that different soils varied in relation to the amount of lime needed to effect partial sterilization. They assumed that each soil absorbed a certain amount before the antiseptic action began.

Miller (*51*) carried out a rather intensive study of the effect of lime and calcium carbonate in both calcium-rich and calcium-poor soils, and also in soils low and high in organic matter. His results were in accordance with those obtained by many other investigators—namely, that lime in small amounts increased the number of bacteria, while larger application, decreased at first and later increased the number. To this investigator it appeared that lime was a direct stimulant to the soil flora. The same general increase was obtained with soil low and soil high in calcium content, and also in soils low and high in organic matter. Unlike lime, calcium carbonate brought about only a slight increase in the number of bacteria.

Beckwith, Vass, and Robinson (*3*) applied lime at the rate of 2 tons per acre to both acid and nonacid soils. Only the soils with an acid reaction or where large amounts of organic matter were added showed an increase in the number of bacteria from the lime treatment. Under the same conditions ammonification and nitrification responded in a like manner.

Soil was treated with lime, magnesium oxid, and magnesium carbonate by Lemmermann and Fischer (*34*). They found that magnesium oxid caused a greater increase in the number of bacteria than did either lime or magnesium carbonate.

Engberding (*13*) treated clay soil with 0.1 per cent of lime and with 0.5 per cent of magnesium oxid. Both treatments increased the number of bacteria, although the lime seemed to give better results.

Jenkins and Britton (*26*) showed that by using heavy applications of lime with raw-bone meal, the latter was decomposed more rapidly.

Fabricius and Van Feilitzen (*14*) noted an increase in the number of bacteria in moor soils treated with lime.

Lemmermann, Fischer, Kappen, and Blanck (*35*) reported a gain in the number of bacteria in cultivated and noncultivated moor soils and in clay soils when lime or calcium carbonate was added. However, an increase in the number of bacteria was not shown in a sandy clay soil or in a sandy soil.

AMMONIA AND NITRATES

Withers and Fraps (*62, 63*) added calcium carbonate to soil treated with nitrogenous material and observed a gain in nitrates which was greater in the calcium-carbonate soil than in the control. Koch (*31*) reported similar results by applying lime. Fraps (*19*) treated acid and nonacid soils with calcium and magnesium carbonates and noted an increase in nitrates in all cases, especially in the acid soil. However, calcium carbonate gave better results than were obtained with magnesium carbonate.

By applying calcium carbonate to a soil treated with ammonium sulphate, Lemmermann, Blanck, Heinitz, and Von Wlodeck (*36*) noticed a loss in ammonia. Lipman, Brown, and their associates (*4, 5, 42, 43*) studied the effect of calcium carbonate on the actitivies of soil microorganisms. Lipman and Brown (*44*) showed an increase in ammonification with monocalcium phosphate [$CaH_4(PO_4)_2$] and dicalcium phosphate [$Ca_2H_2(PO_4)_2$], but a decrease with tricalcium phosphate [$Ca_3(PO_4)_2$] when applied to soil.

Wohltmann, Fischer, and Schneider (*64*) inoculated liquid media with soil treated with magnesium oxid and lime and observed an increase in both ammonification and nitrification. Hutchinson (*22*) observed that in liquid cultures magnesium carbonate retarded nitrification in practically every case, while calcium carbonate had but little influence on this process.

Vogel (*57*) inoculated a nitrogenous solution containing calcium carbonate with a soil suspension and obtained an increase in ammonia. A similar test for nitrification was made, but an increase in nitrates was not obtained. Somewhat similar results were obtained by Paterson and Scott (*53*). Hutchinson and McLennan (*25*) reported that calcium carbonate treatments caused a slight increase in nitrification in soil.

Greaves (*21*), working with a Utah soil high in lime and magnesium oxid, found that calcium carbonate in all concentrations increased the formation of ammonia, while magnesium carbonate retarded ammonification except when applied in very small amounts. The chlorids of magnesium and calcium appeared to be very toxic. With a Japanese soil Machida (*48*), of the Japanese Experiment Station at Tokio, noted that calcium chlorid retarded ammonification, while magnesium chlorid increased it. He found that nitrification was favored more by the use of magnesium carbonate than by calcium carbonate.

The work of McBeth and Wright (*49*) showed that the chlorids, the sulphates, and especially the carbonates inhibited nitrification. Lyon and Bizzell (*47*) reported that 10 days after treatment with lime the number of bacteria was doubled.

Fred and Graul (*20*) concluded that the accumulation of nitrates from casein or gelatin in acid soils was not materially benefited by calcium carbonate. In many cases in acid soil calcium carbonate increased and

later decreased the nitrate content, and it was assumed that a loss of nitrate nitrogen occurred because of the increase in the total number of microorganisms.

Allen and Bonazzi (*1*) showed that the addition of ground limestone to a noncalcareous soil brought about a more rapid nitrification. Kelley (*28*) studied the effects of calcium and magnesium carbonates on ammonification and nitrification in a California soil high in basic substances. His results showed that calcium carbonate benefited ammonification slightly and nitrification to a great extent, while magnesium carbonate was toxic to both processes. This investigator failed to show any definite ratio of calcium and magnesium which favored the nitrifying and ammonifying power of this soil. With Hawaiian soils, high in lime and magnesium oxid, Kelley (*29*) obtained similar results. Dolomitic and calcareous limestones gave results similar to those obtained with calcium carbonate.

Kellerman and Robinson (*27*) obtained nitrification of ammonium sulphate in a soil with a high magnesium content. When calcium carbonate was added, an increase in nitrification was noted; with magnesium carbonate a decrease, except when the latter substance was added to the soil in very small amounts. Owen (*52*) reported that magnesium carbonate favored nitrification more than calcium carbonate.

White (*61*) and Voorhees and Lipman (*58*) treated soil with pure lime and with a lime containing magnesium. Better nitrification and in most cases better ammonification was obtained from the magnesium than from the nonmagnesium limed soil. Soil treated with magnesium carbonate and inoculated into a liquid medium was shown by Lipman and Brown (*41*) to retard nitrification.

It was shown by Ehrenberg (*12*), Lemmermann, Aso, Fischer, and Fresenius (*37*), and Wheeler, Sargent, and Hartwell (*60*), that when lime or calcium carbonate was applied to soil, the decomposition of organic matter was accelerated.

NITROGEN FIXATION

Fischer (*15, 16*) demonstrated the fact that both lime and magnesium oxid increased the reproduction of *Bacillus azotobacter* in soil. The oxid of magnesium seemed to give better results than lime.

So much is *Bacillus azotobacter* influenced by lime that Christensen and Larsen (*9*) suggested the use of this organism to measure the reaction of soil. In a later publication Christensen (*8*) showed that the growth of this organism in solution took place only when inoculated with a basic soil. In their work with Danish soils, Weis and Bornebusch (*59*) confirmed Christensen's results. Loew (*45*) showed that lime added to a soil increased the growth of the film of *B. azotobacter* formed in liquid cultures. Results similar to Loew's were obtained by Cauda (*6*) when calcium carbonate was used.

Fischer (*16*) obtained a better growth of *Bacillus azotobacter* from limed than from unlimed clay soil. On the other hand, Koch, Litzendorf, Krull, and Alves (*32*) reported that lime retarded free-nitrogen fixation. Krüger (*33*) treated soil with lime and obtained an increase in nitrogen fixation.

Purer and better film growth of *Bacillus azotobacter* was obtained by Ashby (*2*) with magnesium carbonate than with calcium carbonate.

The effect of calcium carbonate and magnesium carbonate on the fixation of nitrogen by *Bacillus azotobacter*, both in liquid cultures and in soil, was studied by Lipman and Burgess (*40*). In every case magnesium carbonate alone proved toxic. These authors observed that when 15 parts of calcium carbonate were mixed with 1 part of magnesium carbonate, the latter was no longer toxic to this organism.

SUMMARY OF LITERATURE

From the citations just given it appears that the addition of calcium and magnesium, either in the form of oxid or carbonate, to soil, and especially to acid soil, brings about conditions favorable to the growth of certain groups of microorganisms. There are many factors which have been given little or no consideration—for instance, what relationship exists between the total number of bacteria in soil and the quantity of soil acid neutralized? With few exceptions, little attention has been directed toward the relative effect of calcium and magnesium carbonates on the soil flora. There exists a diversity of opinion with regard to the relation of bacteria to these two compounds. This lack of harmony may be due to the difference in the soil types which have been studied.

Again, it seems that no one has tried to measure the effect of calcium and magnesium carbonates on pure cultures of bacteria in sterilized acid soil. To obtain information with regard to these points, a series of experiments was planned.

EXPERIMENTAL WORK

For this study three Wisconsin soils, acid Colby silt loam, acid Plainfield sand, and neutral Miami silt loam, were used. The Colby silt loam was collected near Marshfield, the Plainfield sand from Hancock, and the Miami silt loam from Madison. At the laboratory each soil was passed through a 4-mm. sieve and thoroughly mixed.

The percentage composition of these soils is given below:

Constituent.	Colby silt loam.	Miami silt loam.	Plainfield sand.
Potassium	1. 51	2. 16	0. 93
Nitrogen	. 198	. 15	. 09
Phosphorous	. 072	. 15	. 032
Calcium oxid	. 0907	. 185	. 0023
Organic matter	3. 91	2. 74	1. 41

The calcium-carbonate requirement of the acid soils was determined according to the Truog barium-hydroxid method. In calculating the amount of acid in each soil only the active acidity was considered. For every 100 gm. of Colby silt loam on the dry basis 1.05 gm. of calcium carbonate were required to correct the active acidity, and for 100 gm. of Plainfield sand 0.21 gm. Three different bases were used to neutralize the acidity in these soils—namely, pure precipitated calcium carbonate, pure precipitated magnesium carbonate, and commercial ground limestone. The limestone, the analysis of which showed 53 per cent of calcium oxid and 43 per cent of magnesium oxid, was ground to pass through a 100-mesh sieve.

Aside from the compounds just named, monocalcium phosphate was used in certain experiments. The phosphate was employed to find out whether or not the calcium of this phosphate salt would serve in a like capacity as that of calcium carbonate. Accordingly the monocalcium phosphate was added to the soil alone and in various mixtures with calcium carbonate.

The calcium carbonate, magnesium carbonate, and limestone were added in amounts sufficient to satisfy one-fourth, one-half, and full calcium-carbonate requirement—that is, to neutralize one-fourth, one-half, and the total active acidity. The phosphate was added in varying amounts. After the bases and phosphate were thoroughly mixed with the soil, the latter was then poured into earthenware jars and the moisture content raised to one-half saturation with distilled water. At definite intervals samples were drawn and plate counts made. The soil of each jar, after the sample had been drawn, was poured on sterile paper, thoroughly mixed, and returned to the original jar. In order to reduce evaporation and to prevent outside contamination, the jars were covered with cheesecloth. The entire series of jars was incubated in the greenhouse at approximately 22° C.

At regular intervals the effect of these compounds on the total number of bacteria in the soil, on ammonification, and on nitrification was studied.

INFLUENCE OF CALCIUM CARBONATE, LIMESTONE, AND MONOCALCIUM PHOSPHATE ON THE NUMBER OF BACTERIA IN SOIL

Colby silt loam.—Two-kgm. portions of soil were treated as outlined in Table I and incubated for a period of five months. During this time eight plate counts were made with Heyden-Nährstoff agar. These plates were incubated at 27° C. for 10 days. The influence of monocalcium phosphate on the number of bacteria in the soil was tested simultaneously with that of the carbonates. The data of this experiment are given in Table I.

TABLE I.—*Influence of calcium carbonate, limestone, and monocalcium phosphate on the number of bacteria in Colby silt loam*

Treatment.	Number of bacteria in 1 gm. of dry soil.									
	After 1 week.	Relative.	After 2 weeks.	Relative.	After 3 weeks.	Relative.	After 8 weeks.	Relative.	After 20 weeks.	Relative.
None	18,600,000	100	19,200,000	100	12,800,000	100	13,000,000	100	6,700,000	100
One-fourth calcium carbonate	21,000,000	113	22,400,000	116	21,300,000	166	18,000,000	138	16,400,000	245
One-half calcium carbonate	20,500,000	111	26,000,000	135	14,000,000	109	13,000,000	100	14,700,000	220
Full calcium carbonate	18,100,000	97	21,200,000	110	13,100,000	102	18,000,000	138	13,600,000	202
One-fourth limestone	21,000,000	113	24,500,000	127	15,000,000	117	16,000,000	123	13,500,000	201
One-half limestone	25,300,000	136	19,500,000	101	15,500,000	121	14,900,000	114	11,500,000	173
Full limestone	22,300,000	120	18,100,000	94	20,700,000	161	17,100,000	131	12,700,000	189
0.5 gm. monocalcium phosphate	14,300,000	77	15,500,000	80	18,000,000	148	13,500,000	103	6,400,000	95
2 gm. monocalcium phosphate	22,300,000	120	21,000,000	109	20,200,000	157	13,600,000	104	8,300,000	124
0.5 gm. monocalcium phosphate + one-fourth calcium carbonate	16,000,000	86	15,000,000	78	18,300,000	142	17,200,000	132	17,400,000	265
0.5 gm. monocalcium phosphate + full calcium carbonate	21,200,000	114	31,700,000	165	22,500,000	175	15,400,000	119	14,300,000	213
2 gm. monocalcium phosphate + one-fourth calcium carbonate	14,200,000	76	21,100,000	109	26,200,000	204	15,200,000	117	11,600,000	173
2 gm. monocalcium phosphate + full calcium carbonate	16,100,000	86	20,900,000	108	35,600,000	278	12,500,000	97	11,000,000	164

It will be seen from the figures in Table I that in practically every case calcium carbonate, either pure or in the form of limestone (dolomitic), increased the growth of bacteria to a considerable extent. As compared with the untreated soil, the favorable influence of the calcium compounds on the number of bacteria was greatest 3, 8, and 20 weeks after treatment. Apparently these compounds of calcium, especially the carbonate, have little influence on the soil flora for the first week. This is to be expected, since calcium carbonate is very slowly soluble.

The most striking fact noted from the results of this experiment is the marked stimulation of the microorganisms following small applications of calcium carbonate. Figure 1 is a diagram showing the effect of calcium carbonate and limestone on the total number of bacteria. One-fourth enough calcium carbonate to neutralize the entire soil acidity, with only one exception, showed the greatest increase in the number of bacteria. If grouped according to their effect on the number of soil organisms, one-fourth calcium carbonate gave the greatest gain in the number of bacteria, one-half, the next greatest, and full, the least. The beneficial effect of calcium carbonate extended over the entire period of five months—that is, the treated soil gave a decided increase in the total number of bacteria as compared with the untreated soil. In general, pure calcium carbonate proved superior to limestone in its effect on the bacteria of Colby silt loam soil. This superiority of calcium carbonate

as compared with limestone was due probably to the difference in solubility of the two compounds; pure calcium carbonate is more soluble than the dolomitic limestone.

The monocalcium phosphate in small amounts apparently did not increase the total number of bacteria, whereas in larger amounts, applied alone, it was slightly beneficial. In two instances a combination of calcium carbonate and phosphate showed an increase in the total number of microorganisms. However, in most cases the increase was no greater than that obtained with calcium carbonate alone.

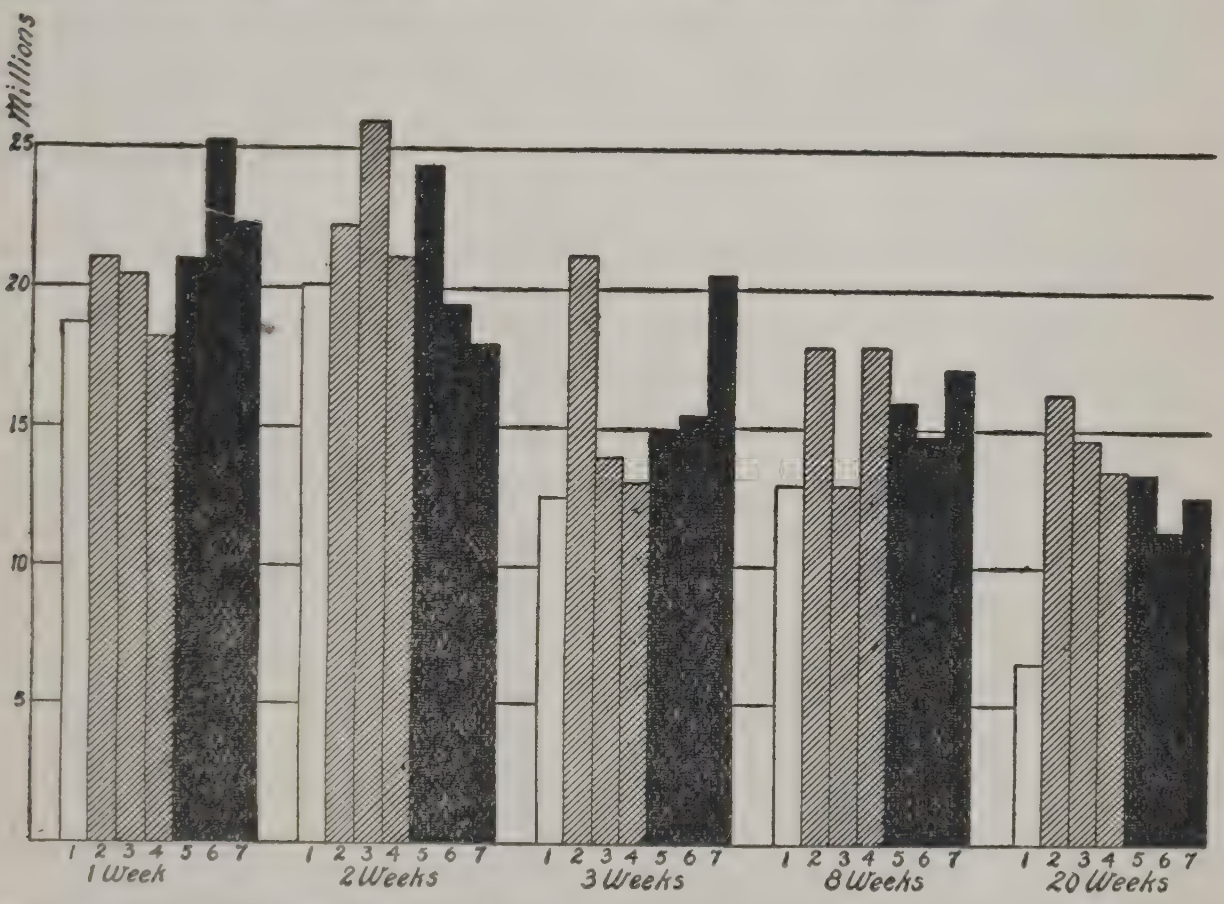

FIG. 1.—Diagram showing the influence of calcium carbonate and limestone on the number of bacteria in Colby silt loam.

1=no treatment.
2=one-fourth cacium carbonate.
3=one-half calcium carbonate.
4=full calcium carbonate.
5=one-fourth limestone.
6=one-half limestone.
7=full limestone.

A comparison of the influence of calcium from calcium phosphate with that from calcium carbonate on the number of bacteria in Colby silt loam is shown in figure 2. Although there are many fluctuations, the results indicate that calcium phosphate alone or with calcium carbonate increases the number of bacteria much sooner than does the carbonate alone. Here the maximum gain with the phosphate was noted 3 weeks after treatment instead of 20 weeks, as in the case of calcium carbonate.

PLAINFIELD SAND.—The preceding experiment was repeated with a Plainfield sand, a soil very low in organic matter. The results obtained in this test are presented in Table II.

TABLE II.—*Influence of calcium carbonate, limestone, and monocalcium phosphate on the number of bacteria in Plainfield sand*

Treatment.	Number of bacteria in 1 gm. of dry soil.									
	After 1 week.	Relative.	After 2 weeks.	Relative.	After 3 weeks.	Relative.	After 8 weeks.	Relative.	After 20 weeks.	Relative.
None	10,300,000	100	3,500,000	100	5,500,000	100	4,700,000	100	2,450,000	100
One-fourth calcium carbonate	10,000,000	97	3,600,000	102	6,000,000	109	6,600,000	140	4,850,000	197
One-half calcium carbonate	6,600,000	64	4,660,000	130	11,200,000	203	7,700,000	164	4 330,000	172
Full calcium carbonate	6,000,000	60	5,520,000	158	18,300,000	332	7,300,000	155	5,800,000	236
One-fourth limestone	5,800,000	56	4,660,000	130	11,000,000	200	8,500,000	181	4,000,000	161
One-half limestone	8,000,000	77	3,800,000	109	10,000,000	180	7,700,000	164	3,000,000	122
Full limestone	9,700,000	94	4,200,000	120	9,700,000	176	7,200,000	154	4,530,000	184
0.5 gm. monocalcium phosphate	8,500,000	84	4,200,000	120	5,700,000	103	4,100,000	87	2,650,000	108
2 gm. monocalcium phosphate	5,000,000	48	4,200,000	120	8,900,000	161	3,200,000	68	2,000,000	82
0.5 gm. monocalcium phosphate + one-fourth calcium carbonate	16,000,000	156	3,100,000	90	14,500,000	263	8,000,000	170	2,120,000	86
0.5 gm. monocalcium phosphate + full calcium carbonate	12,000,000	116	4,200,000	120	9,400,000	170	6,800,000	144	7,770,000	317
2 gm. monocalcium phosphate + one-fourth calcium carbonate	11,200,000	109	2,700,000	80	9,300,000	169	6,600,000	140	3,410,000	139
2 gm. monocalcium phosphate + full calcium carbonate	5,700,000	55	5,200,000	150	10,300,000	187	8,100,000	172	3,660,000	148

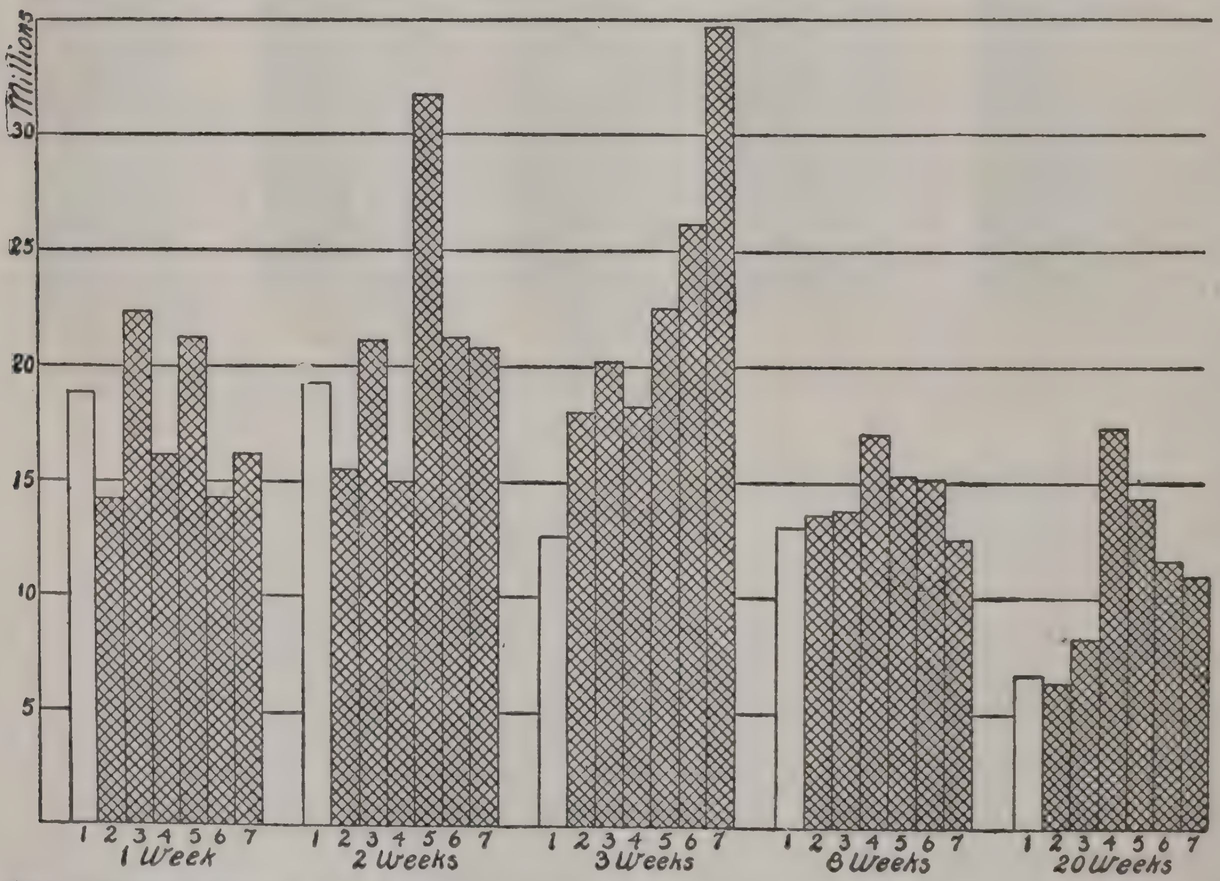

FIG. 2.—Diagram showing the influence of calcium carbonate and monocalcium phosphate on the number of bacteria in Colby silt loam.

1=no treatment.

2=0.5 gm. monocalcium phosphate.

3=2 gm. monocalcium phosphate.

4=0.5 gm. monocalcium phosphate +one-fourth calcium carbonate.

5=0.5 gm. monocalcium phosphate+full calcium carbonate.

6=2 gm. monocalcium phosphate+one-fourth calcium carbonate.

7=2 gm. monocalcium phosphate+full calcium carbonate.

The results reported in Table II differ somewhat from those obtained with Colby silt loam. One week after the treatment there was a decrease in the number of bacteria in the soil treated with both the calcium carbonate and limestone. However, after the first week the treated soil showed an increase in the number of bacteria.

In contrast with the results of the Colby silt-loam experiment, the Plainfield sand, to which one-fourth calcium carbonate or limestone was added, did not give any marked gain in the total number of bacteria after 1 and 2 weeks. After 3, 8, and 20 weeks, one-fourth calcium carbonate caused a slight increase in the number of soil organisms. It is evident that one-half or full neutralization of the soil acids by the calcium carbonate was required to give the greatest increase in the number of bacteria (fig. 3).

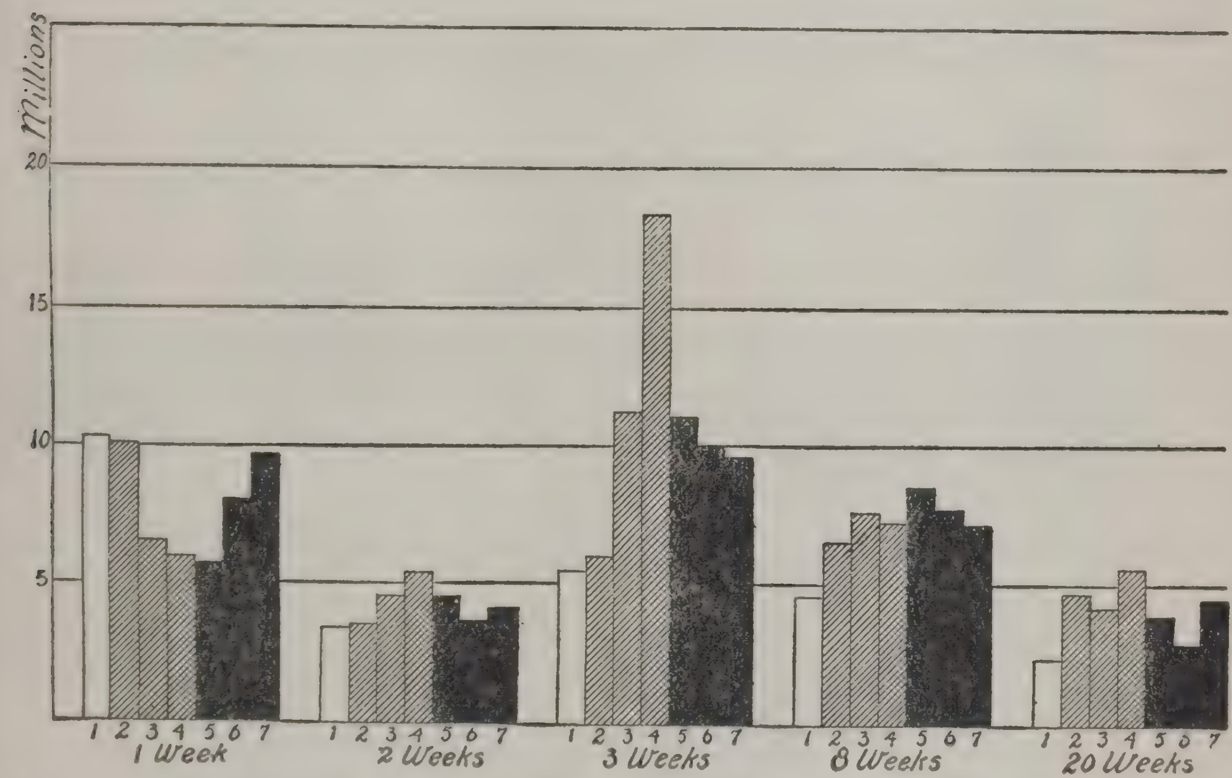

FIG. 3.—Diagram showing the influence of calcium carbonate and limestone on the number of bacteria in Plainfield sand.

1=no treatment.
2=one-fourth calcium carbonate.
3=one-half calcium carbonate.
4=full calcium carbonate.
5=one-fourth limestone.
6=one-half limestone.
7=full limestone.

In general, light applications of limestone gave a greater increase in the number of microorganisms than did calcium carbonate. A difference in the nature of the soil acid in Colby and Plainfield sand probably accounts for the difference in quantity of calcium carbonate required to stimulate the reproduction of bacteria.

Where monocalcium phosphate was added alone to the sandy soil, practically no increase in the number of bacteria was obtained. A combination of the phosphate with calcium carbonate apparently did not stimulate the multiplication of bacteria any better than did calcium carbonate alone except where a combination of 0.5 gm. of monocalcium

phosphate and one-fourth calcium carbonate requirement was used. It is surprising that this combination should favor the development of bacteria more than a heavier application of the same combination (fig. 4).

INFLUENCE OF MAGNESIUM CARBONATE ON THE NUMBER OF BACTERIA IN SOIL

COLBY SILT LOAM.—Since pure calcium carbonate or dolomitic limestone failed to give a large increase in the total number of bacteria, an attempt was made to determine what effect magnesium carbonate would have on the soil flora. Accordingly an experiment was planned in which pure magnesium carbonate was added to the soil. The magnesium car-

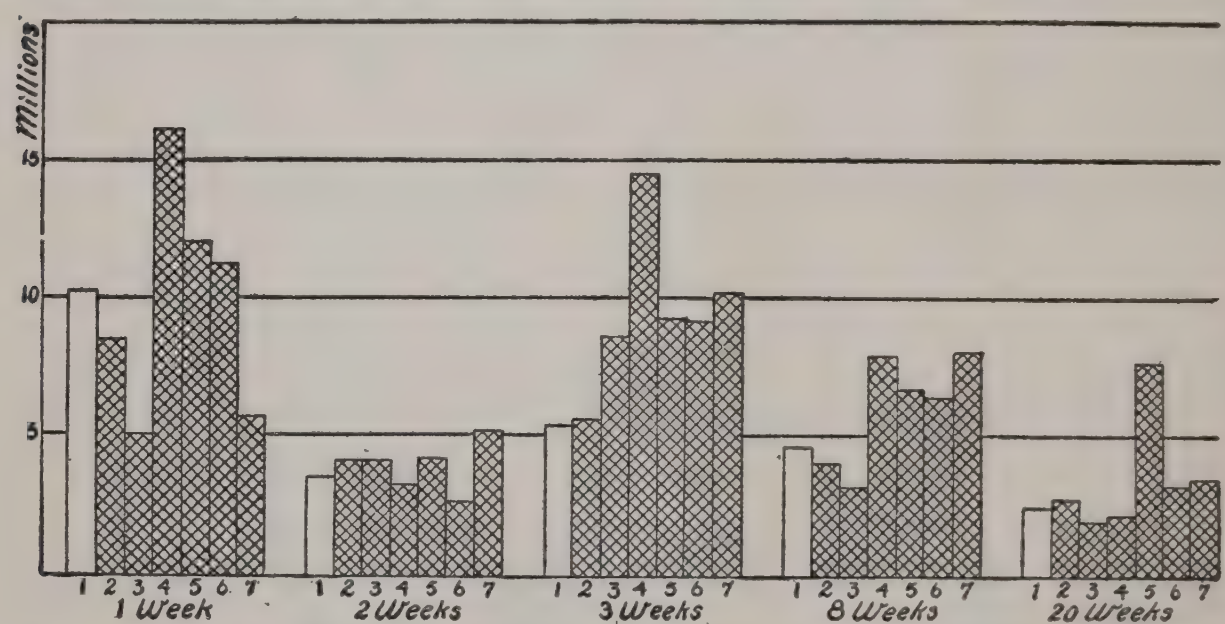

FIG. 4.—Diagram showing the influence of calcium carbonate and monocalcium phosphate on the number of bacteria in Plainfield sand.

1=no treatment.
2=0.5 gm. monocalcium phosphate.
3=2 gm. monocalcium phosphate.
4=0.5 gm. monocalcium phosphate+one-fourth calcium carbonate.
5=0.5 gm. monocalcium phosphate+full calcium carbonate.
6=2 gm. monocalcium phosphate+one-fourth calcium carbonate.
7=2 gm. monocalcium phosphate+full calcium carbonate.

bonate was applied to the soil in the gram-molecular equivalent of calcium carbonate. The procedure in this experiment was similar to that just described. The results of this test are presented in Table III.

TABLE III.—*Influence of magnesium carbonate on the number of bacteria in Colby silt loam*

Treatment.	Number of bacteria in 1 gm. of dry soil.									
	After 1 week.	Relative.	After 2 weeks.	Relative.	After 3 weeks.	Relative.	After 8 weeks.	Relative.	After 20 weeks.	Relative.
None	25,200,000	100	21,000,000	100	19,300,000	100	14,000,000	100	8,600,000	100
One-fourth magnesium carbonate	36,600,000	145	29,700,000	141	30,500,000	157	17,200,000	122	11,700,000	136
One-half magnesium carbonate	44,800,000	178	41,000,000	195	45,200,000	234	19,400,000	138	12,200,000	141
Full magnesium carbonate	156,000,000	615	125,000,000	595	74,500,000	386	59,100,000	422	26,000,000	302

Unlike calcium carbonate and limestone, full applications of magnesium carbonate increased the number of bacteria far beyond the increase obtained with the one-half and one-fourth treatments. From the figures in this table it will be seen that the effect of the application of magnesium carbonate to Colby soil invariably increased the reproduction of the soil bacteria, especially during the first, second, and third week.

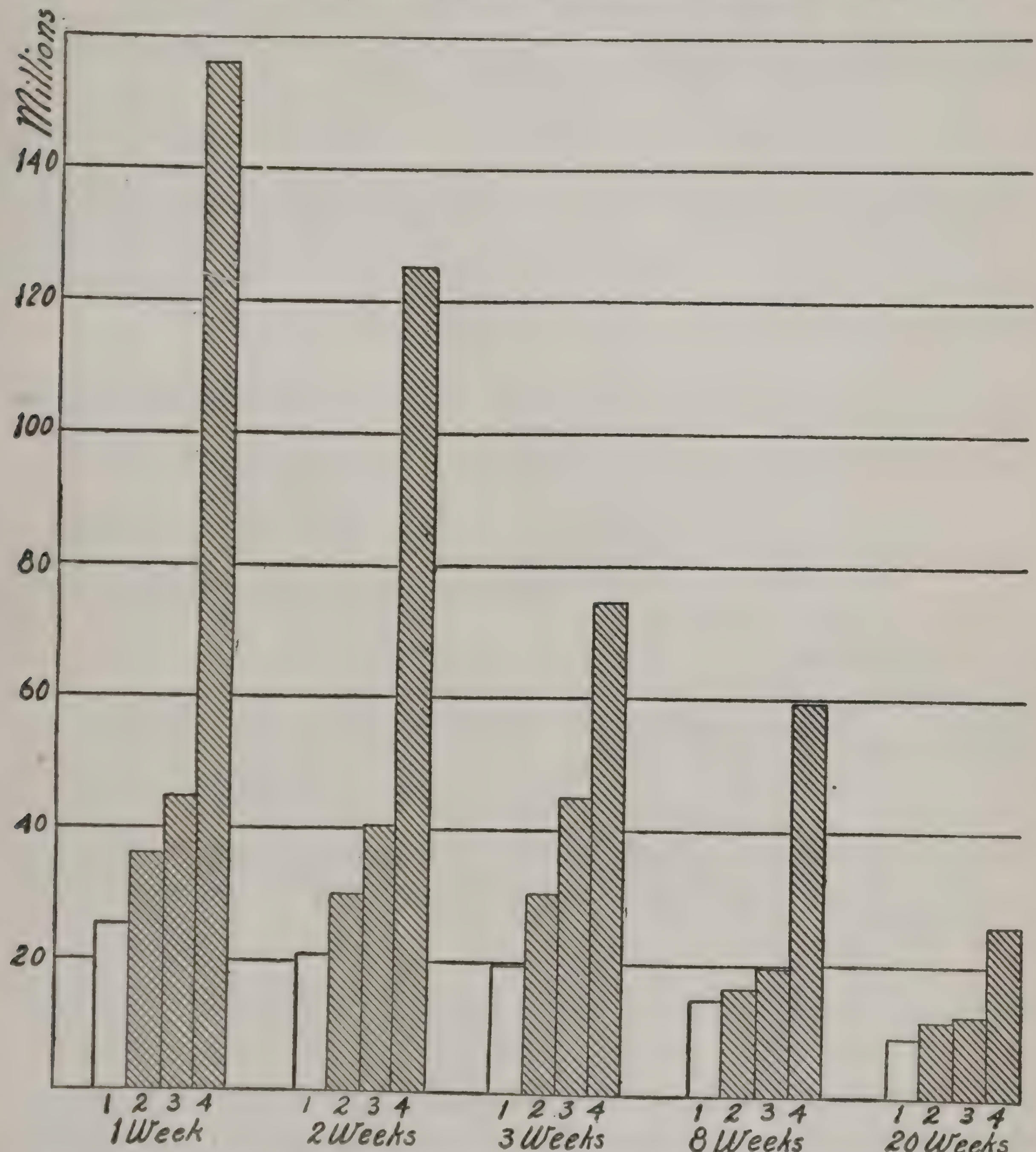

FIG. 5.—Diagram showing the influence of magnesium carbonate on the number of bacteria in Colby silt loam.

1=no treatment.
2=one-fourth magnesium carbonate.
3=one-half magnesium carbonate.
4=full magnesium carbonate.

Figure 5 shows very conclusively the marked effect of magnesium carbonate on the number of soil bacteria. For instance, the increase with full magnesium-carbonate treatment was more than six times as great as that of the control. The absolute numbers varied with the time of the count, but the ratio of numbers between the different quantities of magnesium carbonate remained almost the same throughout the entire

period of 20 weeks. The increase was maintained for a period of 20 weeks, but the total number of organisms decreased greatly during this time.

PLAINFIELD SAND.—It was next arranged to determine the effect of magnesium carbonate on the number of microorganisms in Plainfield sand. The results are summarized in Table IV.

Here again, magnesium carbonate caused a striking increase in the number of soil microorganisms. The results are very similar to those obtained with magnesium carbonate in Colby soil. An enormous increase in the number of soil organisms was noted after 1 and 3 weeks. The sudden drop in numbers after the 3-week period was due probably to a mistake in weighing which resulted in a low moisture content. After the 8- and 20-week periods, the gradual decrease in the number of bacteria was observed, which agrees with the results obtained in Colby silt loam (fig. 6).

TABLE IV.—*Influence of magnesium carbonate on the number of bacteria in Plainfield sand*

Treatment.	Number of bacteria in 1 gm. of dry soil.									
	After 1 week.	Relative.	After 2 weeks.	Relative.	After 3 weeks.	Relative.	After 8 weeks.	Relative.	After 20 weeks.	Relative.
None	10,300,000	100	3,500,000	100	5,500,000	100	4,700,000	100	2,450,000	100
One-fourth magnesium carbonate	21,100,000	203	5,100,000	145	10,400,000	190	5,800,000	123	3,560,000	145
One-half magnesium carbonate	13,500,000	131	5,300,000	151	19,300,000	350	10,300,000	219	5,620,000	228
Full magnesium carbonate	73,300,000	711	8,100,000	230	58,300,000	1,060	16,000,000	340	6,530,000	266

The results secured with these two soil types show clearly that magnesium carbonate in certain soils is a potent factor in the reproduction of soil bacteria.

INFLUENCE OF A MIXTURE OF CALCIUM AND MAGNESIUM CARBONATE ON THE NUMBER OF BACTERIA IN SOIL

In every case magnesium carbonate gave a much greater increase in the number of bacteria than did calcium carbonate. Therefore the question which suggests itself is, What effect will a mixture of calcium and magnesium carbonates have on the soil flora?

It was shown by many investigators, principally Loew (*46*) and his associates, that a soil should contain calcium and magnesium in a certain ratio in order to secure the best plant growth. On the other hand, Lipman (*38*) made the following statement:

> In their behavior toward salts, bacteria differ in some respects from both plants and animals and occupy a position by themselves.

From his study of *B. subtilis*, he concluded that no antagonism exists between calcium and magnesium. In a later publication Lipman (*39*) gave an extensive review of the literature bearing on the subject of the

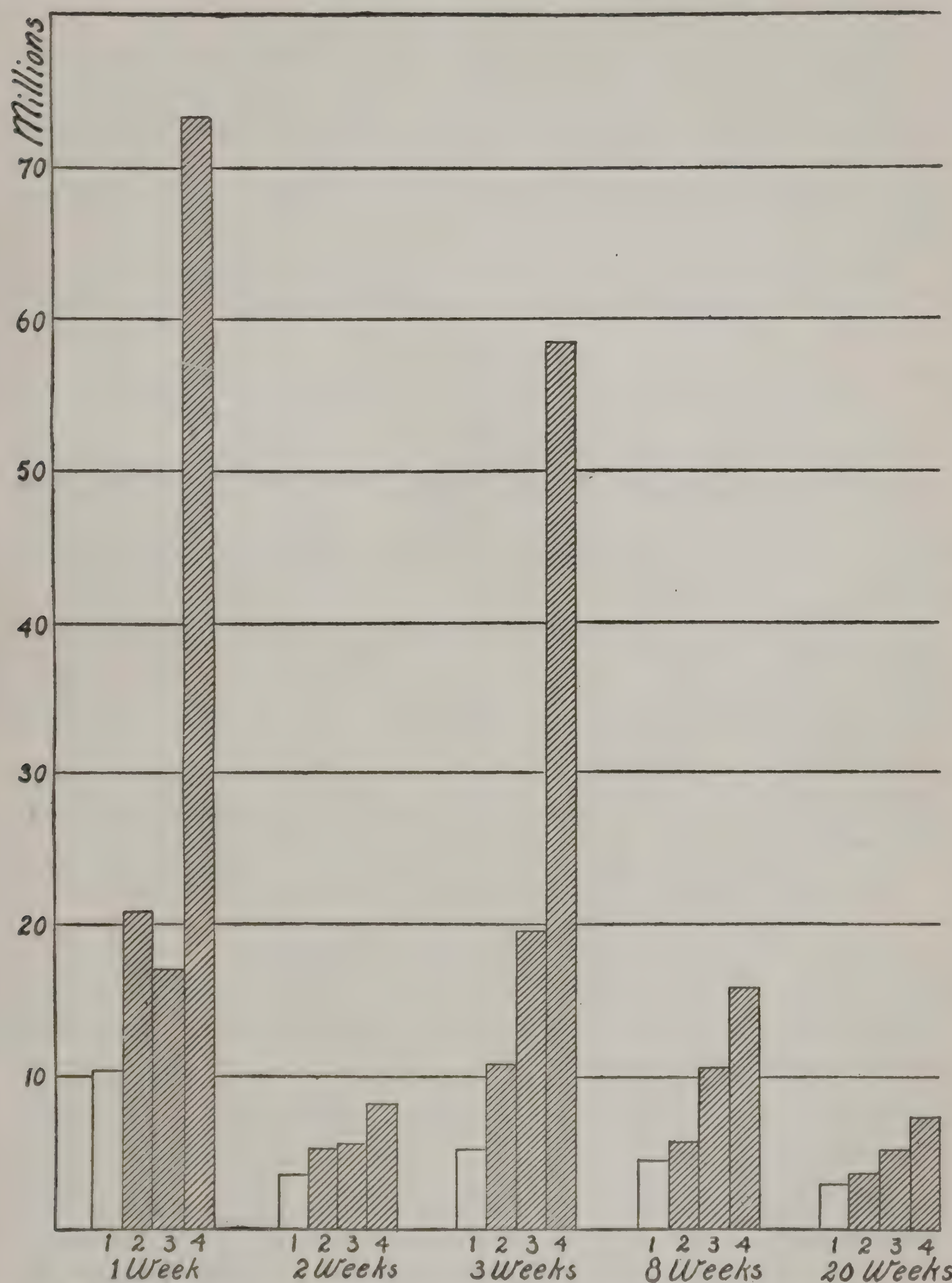

FIG. 6.—Diagram showing the influence of magnesium carbonate on the number of bacteria in Plainfield sand.

1=no treatment.
2=one-fourth magnesium carbonate.
3=one-half magnesium carbonate.
4=full magnesium carbonate.

proper lime-magnesia ratio in soils. He concluded that there is little evidence to support this hypothesis of the lime-magnesia ratio. In accord with Lipman's results, certain investigators (*28*, *29*) showed that various

mixtures of calcium and magnesium carbonates are not favorable to the development of soil bacteria.

To study the effect of a mixture of calcium and magnesium carbonates on the total number of bacteria in both Colby silt loam and Plainfield sand, a series of tests was made. This mixture was employed in amounts equivalent to the neutralizing power of calcium carbonate—that is, enough of the mixture was added to neutralize one-fourth, one-half, and all of the soil acids. Each mixture was made by adding the carbonates in gram-molecular equivalent amounts. The results are presented in Table V.

TABLE V.—*Influence of a mixture of calcium and magnesium carbonates on the number of bacteria in Colby silt loam and Plainfield sand*

COLBY SILT LOAM

Treatment.	Number of bacteria in 1 gm. of dry soil.									
	After 1 week.	Relative.	After 2 weeks.	Relative.	After 3 weeks.	Relative.	After 8 weeks.	Relative.	After 20 weeks.	Relative.
None	19,200,000	100	27,600,000	100	27,500,000	100	14,200,000	100	8,700,000	100
One-fourth: One-half calcium carbonate, One-half magnesium carbonate.	36,500,000	190	29,000,000	105	27,500,000	100	20,700,000	145	15,100,000	173
One-half: One-half calcium carbonate, One-half magnesium carbonate.	14,700,000	76	32,500,000	117	28,600,000	104	17,000,000	120	12,200,000	140
Full: One-half calcium carbonate, One-half magnesium carbonate.	31,800,000	165	45,300,000	163	44,500,000	162	20,000,000	140	13,300,000	152

PLAINFIELD SAND

Treatment.	After 1 week.	Relative.	After 2 weeks.	Relative.	After 3 weeks.	Relative.	After 8 weeks.	Relative.	After 20 weeks.	Relative.
None	10,300,000	100	3,500,000	100	5,000,000	100	4,700,000	100	2,450,000	100
One-fourth: One-half calcium carbonate, One-half magnesium carbonate.	11,300,000	109	3,200,000	91	11,700,000	234	5,500,000	117	4,250,000	173
One-half: One-half calcium carbonate, One-half magnesium carbonate.	11,800,000	115	3,800,000	109	13,300,000	266	8,400,000	180	3,500,000	143
Full: One-half calcium carbonate, One-half magnesium carbonate.	13,100,000	127	5,000,000	143	18,400,000	370	8,600,000	184	2,620,000	107

It will be seen from the figures of the table that a mixture of calcium and magnesium carbonates increased the number of bacteria in Colby silt loam and Plainfield sand. The most interesting fact ascertained from this test is that magnesium carbonate plus calcium carbonate is less efficient in its effect on the reproduction of bacteria than the equivalent weight of magnesium alone.

INFLUENCE OF CALCIUM CARBONATE, CALCIUM CHLORID, MAGNESIUM CARBONATE, MAGNESIUM CHLORID, DIBASIC MAGNESIUM PHOSPHATE, AND MONOCALCIUM PHOSPHATE ON THE BACTERIA IN SOIL

COLBY SILT LOAM.—Since the increase in number of bacteria in soil treated with magnesium carbonate seemed far too great to be accounted for by the correction of the soil acidity alone, an experiment was made to study the effect of a neutral salt of magnesium, and a magnesium phosphate, on the number of bacteria in Colby silt loam soil. Two points were considered in planning this test: First, the action of the magnesium and calcium ions on the bacteria; and second, the possibility of the combining of the calcium or magnesium, especially the latter, with the phosphate of the soil, thus liberating the phosphate in a more available form.

It was reported by Truog (*56*) that magnesium phosphate favors the phosphorus assimilation by plants more than any other phosphate. If this be true, then the bacteria (lower plant life) should be favored by a phosphate when in this form. Therefore the question arises, Does the magnesium carbonate when added to soil react with the phosphates to form magnesium phosphate?

In order to make these tests, magnesium carbonate, magnesium chlorid, dibasic magnesium phosphate, also calcium carbonate, calcium chlorid, and monocalcium phosphate were added to the soil alone and in various mixtures.

TABLE VI.—*Influence of calcium carbonate, calcium chlorid, magnesium carbonate, magnesium chlorid, dibasic magnesium phosphate, and monocalcium phosphate on the bacteria of Colby silt loam*

Treatment.	Number of bacteria in 1 gm. of dry soil.							
	After 1 week.	Relative.	After 2 weeks.	Relative.	After 3 weeks.	Relative.	After 8 weeks.	Relative.
None	28,000,000	100	19,000,000	100	23,200,000	100	12,200,000	100
0.1 per cent magnesium chlorid	16,000,000	57	22,100,000	116	14,000,000	60	17,700,000	145
0.1 per cent calcium chlorid	18,200,000	65	17,600,000	92	27,500,000	118	10,500,000	86
Full calcium carbonate	89,100,000	318	37,600,000	197	43,300,000	186	26,200,000	214
Full magnesium carbonate	165,000,000	590	121,100,000	637	200,000,000	862	58,700,000	481
0.1 per cent dibasic magnesium phosphate	30,500,000	108	13,100,000	67	14,000,000	60	15,600,000	127
0.1 per cent monocalcium phosphate	38,000,000	135	9,300,000	47	24,000,000	103	11,600,000	95
0.1 per cent dibasic magnesium phosphate+full calcium carbonate	74,100,000	264	66,100,000	347	33,100,000	141	26,000,000	213
0.1 per cent monocalcium phosphate+full calcium carbonate	38,000,000	135	47,300,000	249	46,200,000	199	31,500,000	258
0.1 per cent dibasic magnesium phosphate+full magnesium carbonate	191,000,000	682	190,000,000	1,000	106,000,000	456	85,500,000	700
0.1 per cent monocalcium phosphate + full magnesium carbonate	223,000,000	800	144,000,000	755	197,000,000	849	78,000,000	639

The effect of magnesium phosphate on the number of bacteria was compared with that of magnesium carbonate, calcium phosphate, and calcium carbonate. If the action of the magnesium and calcium carbonates

on the bacteria is derived from the basic part of these compounds, then the salts of these substances—namely, magnesium and calcium chlorid—should increase the number of bacteria in a similar manner. These salts were added in quantities equivalent to 0.1 per cent of the dry weight of the soil. The data for this experiment are presented in Table VI.

The chlorids of calcium and magnesium failed to increase the number of bacteria in Colby silt loam. Instead, the majority of cases showed a loss, especially with calcium chlorid. Calcium and magnesium carbonates, in agreement with a previous study, increased the number of microorganisms. Magnesium carbonate stimulated the development of the bacteria far greater than did calcium carbonate.

When the dibasic magnesium phosphate and monocalcium phosphate were applied alone there did not seem to be any gain in the number of bacteria. The mixtures of monocalcium phosphate and dibasic magnesium phosphate with magnesium carbonate resulted in all except one period in an increase in the number of bacteria beyond that obtained by the use of magnesium carbonate alone. The mixtures made by adding these same phosphates with calcium carbonate did not result in an increase in the number of bacteria beyond that noted where calcium carbonate was used alone.

From the data of this experiment, which are diagramed in figures 7 and 8, it seems safe to conclude that neither the phosphates nor the chlorids of magnesium and calcium alone caused a marked multiplication of the bacteria in Colby silt loam.

Miami silt loam.—A similar test of these different compounds was made with Miami silt loam, a neutral soil. The chlorids of magnesium and calcium were eliminated in this experiment. Since the effect of a carbonate on the number of bacteria in Miami silt loam soil was not known, the carbonates were added in amounts equal to the one-half and full calcium carbonate requirement of Colby silt loam. The data for this experiment are presented in Table VII.

Table VII.—*Influence of calcium carbonate, magnesium carbonate, dibasic magnesium phosphate, and monocalcium phosphate on the number of bacteria in Miami silt loam*

Treatment.	Number of bacteria in 1 gm. of dry soil.					
	After 1 week.	Relative.	After 2 weeks.	Relative.	After 3 weeks.	Relative.
None	10,000,000	100	18,500,000	100	9,600,000	100
One-half calcium carbonate	10,300,000	103	18,500,000	100	13,800,000	143
Full calcium carbonate	13,800,000	138	23,700,000	128	15,100,000	157
One-half magnesium carbonate	17,000,000	170	25,400,000	137	15,500,000	161
Full magnesium carbonate	27,000,000	270	52,400,000	283	21,600,000	225
0.1 per cent monocalcium phosphate	10,600,000	106	20,400,000	110	12,300,000	128
0.1 per cent dibasic magnesium phosphate	15,700,000	157	27,000,000	146	14,300,000	150
0.1 per cent monocalcium phosphate + full calcium carbonate	12,400,000	124	24,000,000	130	13,500,000	140
0.1 per cent dibasic magnesium phosphate + full calcium carbonate	11,100,000	111	20,000,000	108	14,600,000	152
0.1 per cent monocalcium phosphate + full magnesium carbonate	20,000,000	200	35,000,000	189	19,000,000	198
0.1 per cent dibasic magnesium phosphate + full magnesium carbonate	21,600,000	216	43,800,000	236	11,700,000	121

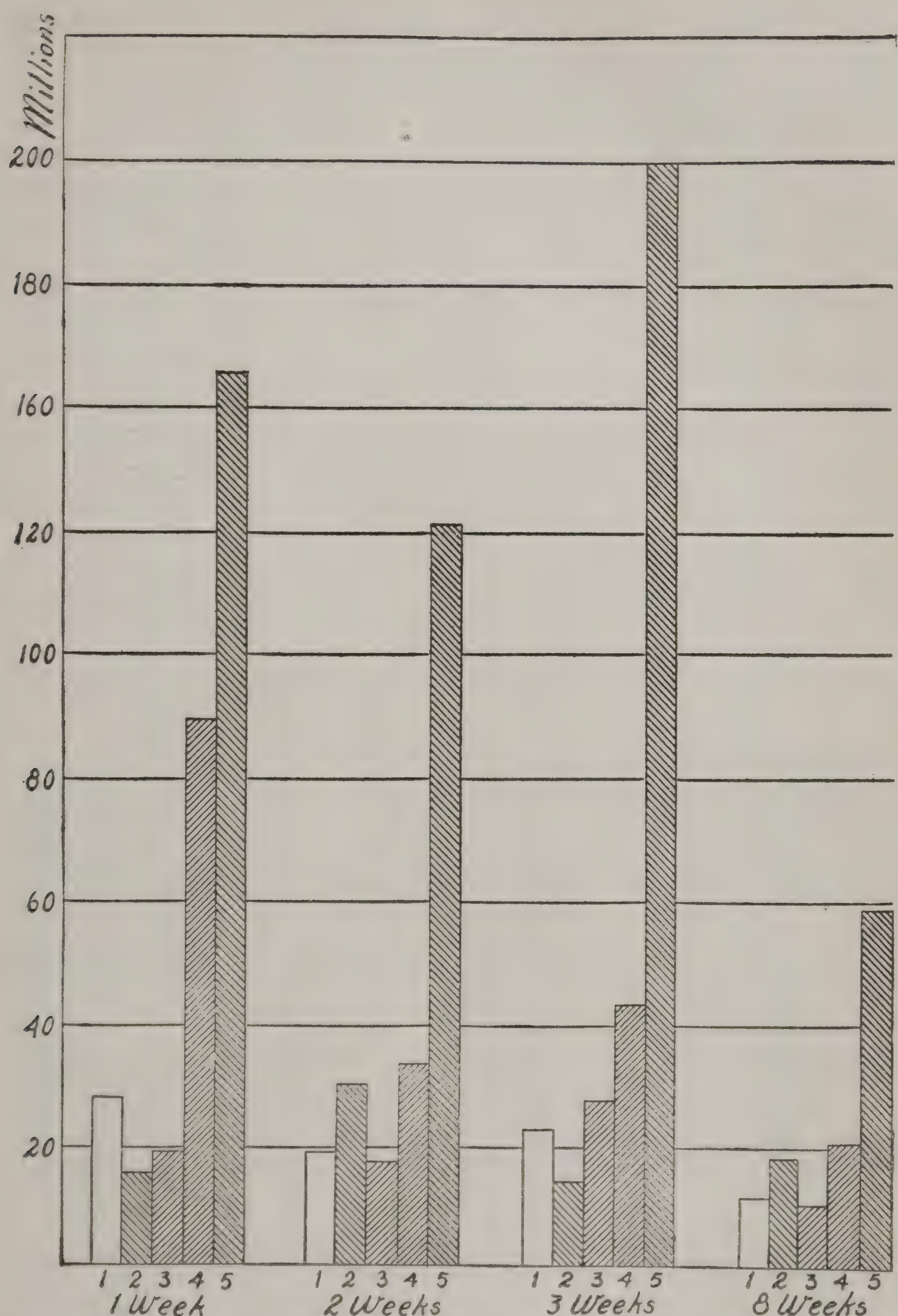

FIG. 7.—Diagram showing the influence of the carbonates and chlorids of magnesium and calcium on the number of bacteria in Colby silt loam soil.

1=no treatment.
2=0.1 per cent magnesium chlorid.
3=0.1 per cent calcium chlorid.
4=full calcium carbonate.
5=full magnesium carbonate.

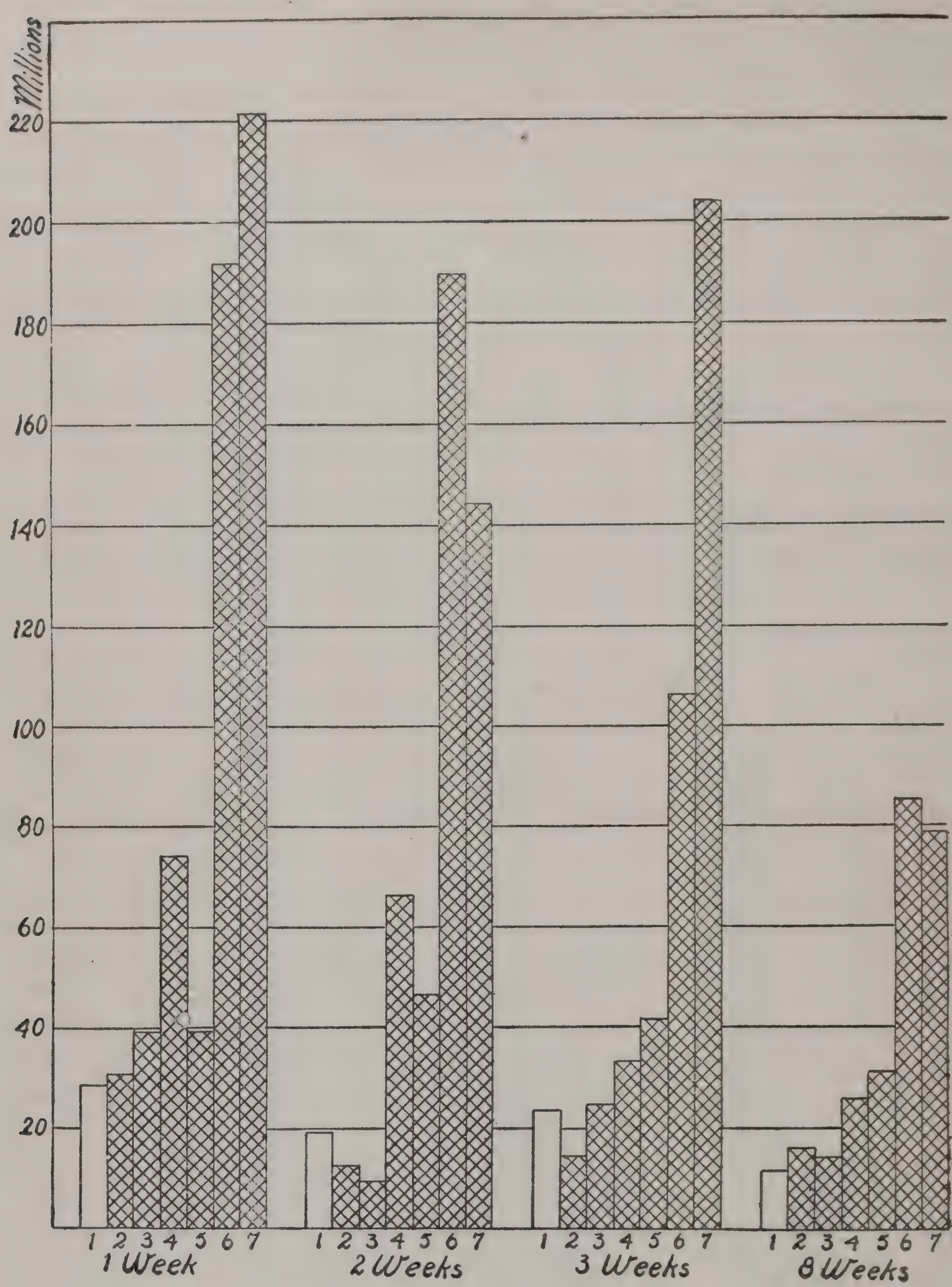

FIG. 8.—Diagram showing the influence of dibasic magnesium phosphate, monocalcium phosphate, calcium carbonate, and magnesium carbonate on the number of bacteria in Colby silt loam.

1=no treatment.
2=0.1 per cent dibasic magnesium phosphate.
3=0.1 per cent monocalcium phosphate.
4=0.1 per cent dibasic magnesium phosphate+full calcium carbonate.
5=0.1 per cent monocalcium phosphate+full calcium carbonate.
6=0.1 per cent dibasic magnesium phosphate+full magnesium carbonate.
7=0.1 per cent monocalcium phosphate+full magnesium carbonate.

From the results which are given in Table VII and from figure 9 it will be seen that both magnesium and calcium carbonate increased the number of bacteria in Miami silt loam soil. Here, again, the increase was much greater with magnesium carbonate than with calcium carbonate. The greatest increase was obtained two weeks after treatment. In all cases, the heavier applications increased the number of bacteria more than did the smaller ones.

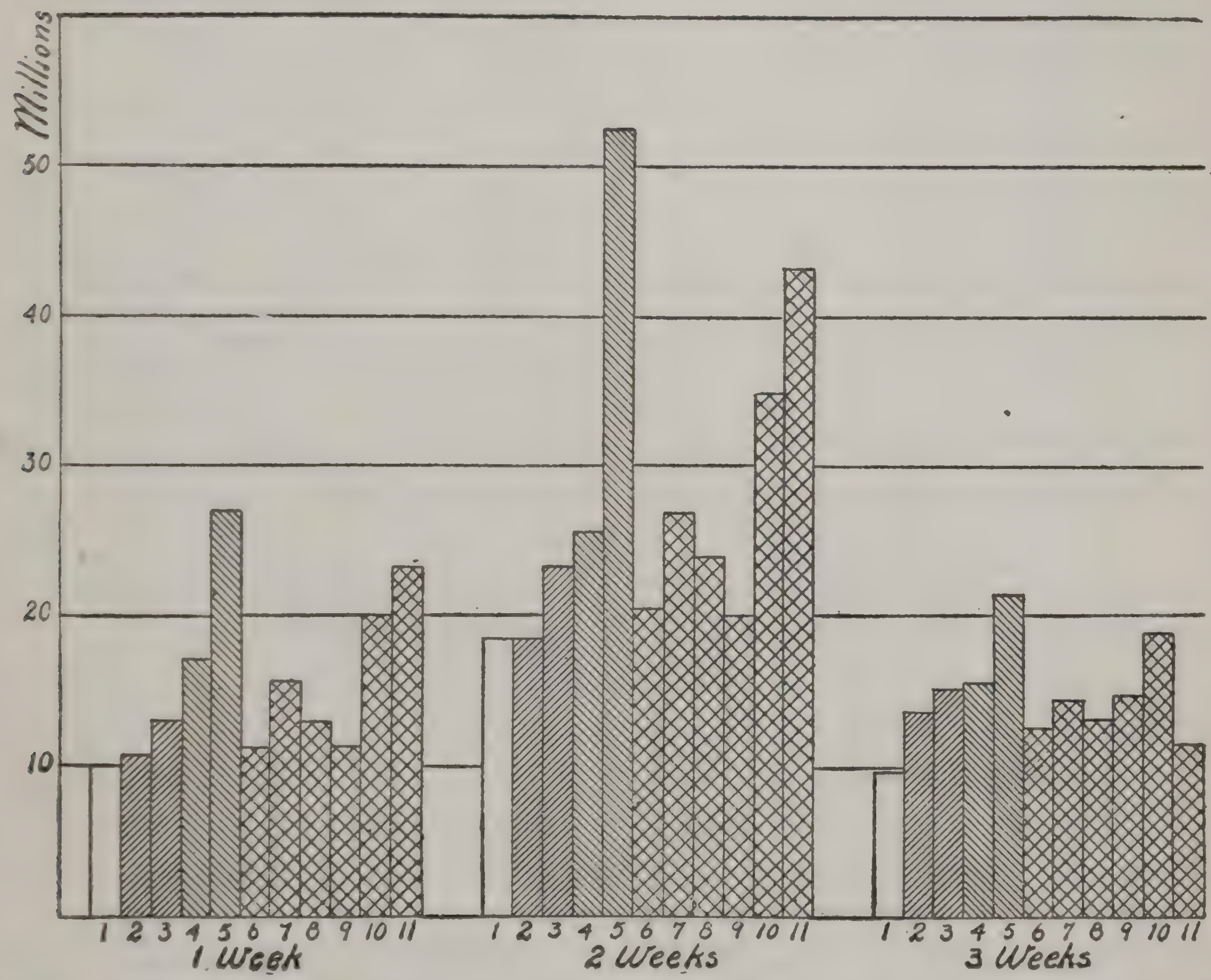

Fig. 9.—Diagram showing the influence of calcium carbonate, magnesium carbonate, dibasic magnesium phosphate, and monocalcium phosphate on the number of bacteria in Miami silt loam.

1=no treatment.
2=one-half calcium carbonate.
3=full calcium carbonate.
4=one-half magnesium carbonate.
5=full magnesium carbonate.
6=0.1 per cent monocalcium phosphate.
7=0.1 per cent dibasic magnesium phosphate.
8=0.1 per cent monocalcium phosphate+full calcium carbonate.
9=0.1 per cent dibasic magnesium phosphate+full calcium carbonate.
10=0.1 per cent monocalcium phosphate +full magnesium carbonate.
11=0.1 per cent dibasic magnesium phosphate+full magnesium carbonate.

The results differentiate themselves from those obtained with acid Colby silt loam soil since the phosphates increased the number of bacteria in the neutral Miami silt loam. It was expected that these phosphates would have a more beneficial effect in neutral than in acid soil, since it is probable that the phosphate was hydrolyzed, thus adding more acid to the soil. In every test magnesium phosphate gave a larger increase in number of bacteria than was obtained by the use of calcium phos-

phate. The dibasic magnesium phosphate increased the number of bacteria to about the same degree as an application of one-half magnesium carbonate. A combination of each phosphate with each carbonate did not prove beneficial in augmenting the number of bacteria in Miami silt loam beyond that caused by each carbonate when used alone. It is evident from the foregoing data, as shown in figure 9, that the relative increase of the number of bacteria from the use of carbonates of calcium and magnesium in Miami silt loam was not so great as when Colby silt loam was treated with the same compounds. However, the phosphate gave a greater relative increase in the neutral Miami silt loam than in the acid Colby silt-loam soil.

INFLUENCE OF MAGNESIUM CARBONATE, CALCIUM CARBONATE, LIMESTONE, AND MONOCALCIUM PHOSPHATE ON THE BACTERIA IN SOIL

Another series of jars was filled with Colby silt loam treated with magnesium carbonate; a second series with the same soil treated with calcium carbonate; a third series with limestone, and a fourth with monocalcium phosphate. All jars were placed in the greenhouse and the soil moisture maintained at half saturation. After one, two, and three months samples of this soil were drawn and tested for ammonifying power, for nitrate content, and for the number of bacteria.

The ammonifying power was determined by mixing 2 per cent of dried blood, which contained 13.4 per cent of nitrogen, with 100 gm. of soil. After adding the dried blood meal, the soil was placed in tumblers, the proper amount of water added, the tumblers covered with petri-dish covers, and incubated at 27° C. for six days. The ammonia was determined by the steam-distillation method.

In order to measure the nitrification in the treated and untreated soil, samples were taken from the jars and the nitrate content determined immediately. This was simply a study of nitrate accumulation in the soil; no nitrogenous substance was added. Nitrates were determined by the phenol-disulphonic acid method. The data for the experiments with Colby silt loam and Plainfield sand are shown in Tables VIII to XI, inclusive.

From the data in Table VIII it will be seen that after one month the increase in the number of bacteria in Colby soil, in conformity with previous tests, was greatest with the magnesium-carbonate treatment. The increase was very marked, about five times greater than that derived from the use of calcium carbonate or limestone. The favorable effect of the magnesium carbonate was noted after both the 2- and 3-month periods. In most cases the calcium carbonate and limestone increased the number of bacteria, notably after two months. The monocalcium phosphate apparently exerted no effect in increasing the

number of bacteria, since it gave no increase except when used in combination with calcium carbonate, and then the increase was no greater than that obtained with calcium carbonate alone.

TABLE VIII.—*Influence of calcium carbonate, magnesium carbonate, limestone, and monocalcium phosphate on the number of bacteria in Colby silt loam*

Treatment.	Number of bacteria in 1 gm. of dry soil.					
	After 1 month.	Relative.	After 2 months.	Relative.	After 3 months.	Relative.
None	14,000,000	100	10,700,000	100	8,000,000	100
One-fourth calcium carbonate	14,370,000	102	13,400,000	125	8,800,000	110
One-half calcium carbonate	12,200,000	87	17,000,000	158	7,100,000	88
Full calcium carbonate	14,370,000	102	14,800,000	138	8,700,000	108
One-fourth limestone	12,200,000	87	17,600,000	164	10,300,000	128
One-half limestone	14,370,000	102	11,200,000	104	8,700,000	108
Full limestone	13,340,000	95	14,300,000	133	10,700,000	133
One-fourth magnesium carbonate	15,180,000	108	11,900,000	111	10,900,000	136
One-half magnesium carbonate	26,000,000	185	25,700,000	240	13,200,000	165
Full magnesium carbonate	101,400,000	724	43,700,000	408	33,500,000	418
0.1 per cent monocalcium phosphate	15,000,000	107	10,700,000	100	8,300,000	103
0.1 per cent monocalcium phosphate + one-fourth calcium carbonate	18,100,000	130	10,800,000	101	7,600,000	95
0.1 per cent monocalcium phosphate + full calcium carbonate	11,380,000	80	13,600,000	127	10,300,000	128

The data in Table IX are in agreement with those obtained with Colby silt loam—that is, magnesium carbonate increased the number of bacteria in Plainfield sand to a considerable extent beyond that produced by calcium carbonate or limestone. The effect of the magnesium carbonate on the number of bacteria was most noticeable one month after the treatment was applied. In general, limestone proved inferior to calcium carbonate in stimulating the number of bacteria in Plainfield sand, except that in one case the monocalcium phosphate when applied alone did not increase the number of bacteria.

TABLE IX.—*Influence of calcium carbonate, magnesium carbonate, limestone, and monocalcium phosphate on the number of bacteria in Plainfield sand*

Treatment.	Number of bacteria in 1 gm. of dry soil.					
	After 1 month.	Relative.	After 2 months.	Relative.	After 3 months.	Relative.
None	6,800,000	100	6,550,000	100	2,100,000	100
One-fourth calcium carbonate	6,220,000	91	5,440,000	83	2,450,000	116
One-half calcium carbonate	6,100,000	90	7,800,000	119	3,200,000	152
Full calcium carbonate	8,770,000	128	8,440,000	128	2,900,000	138
One-fourth limestone	5,600,000	82	5,900,000	90	1,780,000	84
One-half limestone	9,180,000	135	4,900,000	74	2,550,000	121
Full limestone	7,700,000	113	7,660,000	116	2,550,000	121
One-fourth magnesium carbonate	7,240,000	164	5,400,000	82	3,000,000	133
One-half magnesium carbonate	12,640,000	185	7,300,000	111	5,000,000	238
Full magnesium carbonate	44,800,000	658	28,500,000	435	4,800,000	228
0.1 per cent monocalcium phosphate	4,380,000	64	3,000,000	45	2,340,000	111
0.1 per cent monocalcium phosphate+one-fourth calcium carbonate	7,550,000	111	4,670,000	71	3,230,000	154
0.1 per cent monocalcium phosphate+full calcium carbonate	6,620,000	97	4,500,000	68	2,700,000	128

FORMATION OF AMMONIA

The ammonia determinations were made at the end of two and three months. It will be seen from the data in Table X that all treatments accelerated the formation of ammonia from dried blood. After two months monocalcium phosphate plus calcium carbonate showed the greatest effect, although the phosphate-alone treatment gave nearly as good results. Of the carbonates, calcium carbonate appeared to have the greatest effect in increasing the ammonifying power, an effect nearly as great as that obtained from the phosphate applications. Magnesium carbonate exerted a less effect than did calcium carbonate, and limestone had even a less effect than was obtained with magnesium carbonate. After three months the phosphate still retained a lead in stimulating ammonification. Limestone caused a greater increase in ammonia-producing power than did calcium carbonate, while calcium carbonate gave a greater increase than was obtained with magnesium carbonate. From the data on Colby soil it is shown that the increase in ammonifying power is not always parallel with the increase in the total number of organisms.

TABLE X.—*Influence of calcium carbonate, magnesium carbonate, limestone, and monocalcium phosphate on the ammonification of dried blood in Colby silt loam and Plainfield sand*

Treatment.	Ammonia nitrogen in 100 gm. of dry soil.							
	Colby silt loam.				Plainfield sand.			
	After 2 months.	Increase.	After 3 months.	Increase.	After 2 months.	Increase.	After 3 months.	Increase.
	Mgm.	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*
None	37.7	....	51.7	....	49.0	....	55.1	....
One-fourth calcium carbonate	39.0	+ 1.3	55.5	+ 3.8	47.7	− 1.3	52.2	− 2.9
One-half calcium carbonate	....	....	61.6	+ 9.9	40.1	− 8.9	47.0	− 8.1
Full calcium carbonate	53.0	+15.3	62.3	+10.6	40.1	− 8.9	53.1	− 2.0
One-fourth limestone	45.7	+ 8.0	61.6	+ 9.9	51.8	+ 2.8	51.0	− 4.1
One-half limestone	40.6	+ 2.9	64.5	+12.8	38.7	−10.3	51.3	− 3.8
Full limestone	44.2	+ 6.5	69.7	+18.0	37.7	−11.3	73.8	+18.7
One-fourth magnesium carbonate	49.0	+11.3	58.1	+ 6.4	40.3	− 8.7	46.7	− 8.4
One-half magnesium carbonate	51.6	+13.9	53.7	+ 2.0	39.9	− 9.1	47.5	− 7.6
Full magnesium carbonate	57.1	+19.4	52.5	+ 1.8	35.2	−13.8	47.0	− 8.0
0.1 per cent monocalcium phosphate	61.0	+23.3	63.1	+11.4	56.7	+ 7.7	75.3	+20.2
0.1 per cent monocalcium phosphate+one-fourth calcium carbonate	57.5	+19.8	78.0	+26.3	50.5	+ 1.5	73.3	+18.2
0.1 per cent monocalcium phosphate+full calcium carbonate	72.5	+34.8	80.6	+28.9	48.5	− 0.5	65.7	+10.6

The data in Table X for Plainfield sand showed very different results from those obtained with Colby silt loam. After two months a decrease in ammonifying power was noted, except in the case of the one-fourth limestone treatment. The phosphate when applied alone gave the greatest increase, but where calcium carbonate was added in combination with the phosphate, the formation of ammonia was not so great.

With an increase of calcium carbonate there was a decrease in ammonia production. This decrease was shown with all carbonates, magnesium carbonate causing the greatest decrease.

After three months the same order was held by these compounds in stimulating ammonia formation. At this time the full limestone had a greater effect than the smaller limestone applications; the effect was almost as great as that due to the phosphate treatment. It is evident from the results obtained in both Colby silt loam and Plainfield sand, that the greatest accumulation of ammonia (six days' incubation) does not occur where the largest increase in the number of bacteria was obtained. Where the greatest number of bacteria developed, it seems probable that the greatest amount of ammonia should be formed. Since the substances which gave the highest number of bacteria also neutralized the soil acids, it is probable that the ammonia partly escaped through volatilization. Because of the open texture of the Plainfield sand, more ammonia escaped from this soil than from the Colby silt loam.

TABLE XI.—*Influence of calcium carbonate, magnesium carbonate, limestone, and monocalcium phosphate on nitrate accumulation in Colby silt loam and Plainfield sand*

Treatment.	Nitrate nitrogen accumulated in 100 gm. of dry soil.											
	Colby silt loam.						Plainfield sand.					
	After 1 month.	Increase.	After 2 months.	Increase.	After 3 months.	Increase.	After 1 month.	Increase.	After 2 months.	Increase.	After 3 months.	Increase.
	Mgm.	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*
None	5.86	......	6.05	......	6.62	......	1.74	......	1.58	......	1.33	......
One-fourth calcium carbonate	7.23	1.37	6.94	0.89	8.56	1.94	2.42	0.68	1.85	0.27	1.67	0.34
One-half calcium carbonate	6.10	.24	7.56	1.51	9.37	2.75	2.35	.61	2.31	.73	2.55	1.22
Full calcium carbonate	6.25	.37	8.18	2.13	12.00	5.38	2.20	.46	2.22	.64	2.92	1.59
One-fourth limestone	5.53	−.33	6.05	.00	7.25	.63	2.20	.46	1.80	.22	1.73	.40
One-half limestone	5.86	.00	6.40	.35	7.25	.63	1.87	.13	1.67	.09	2.00	.67
Full limestone	6.51	.65	6.42	.37	8.56	.94	1.97	.23	1.87	.29	2.80	1.47
One-fourth magnesium carbonate	6.58	.72	6.75	.70	9.43	2.81	2.10	.36	1.24	−.34	1.50	.17
One-half magnesium carbonate	8.13	2.27	8.44	2.39	12.00	5.38	2.20	.46	2.22	.64	2.70	1.37
Full magnesium carbonate	13.28	7.42	13.00	6.95	15.00	8.38	2.34	.60	2.78	1.20	4.62	3.29
0.1 per cent monocalcium phosphate	5.63	−.23	6.05	.00	7.25	.63	2.12	.38	1.85	.27	3.08	1.75
0.1 per cent monocalcium phosphate + one-fourth calcium carbonate	6.10	.24	6.42	.37	9.37	2.75	2.35	.61	2.41	.83	2.20	.87
0.1 per cent monocalcium phosphate + full calcium carbonate	6.73	.87	8.18	2.13	11.25	4.63	2.80	.06	1.80	.22	3.08	1.75

ACCUMULATION OF NITRATES

From the data presented in Table XI it will be seen that nitrates accumulated faster in both the treated Colby silt loam and Plainfield sand than in the untreated. In both soils the magnesium carbonate benefited nitrification more than did the other carbonates. Next in order to magnesium carbonate was calcium carbonate, and lastly, lime-

stone, in increasing the nitrate content of Colby silt loam and Plainfield sand; the heavier applications gave the highest nitrate accumulation. In the case of the magnesium carbonate with Colby silt loam soil, the increased accumulation reached as high as 8 mgm. The increase in nitrate in the Plainfield sand ran parallel with that in the Colby silt loam where similarly treated, though the accumulation in the sand was much smaller, since this soil is low in organic matter.

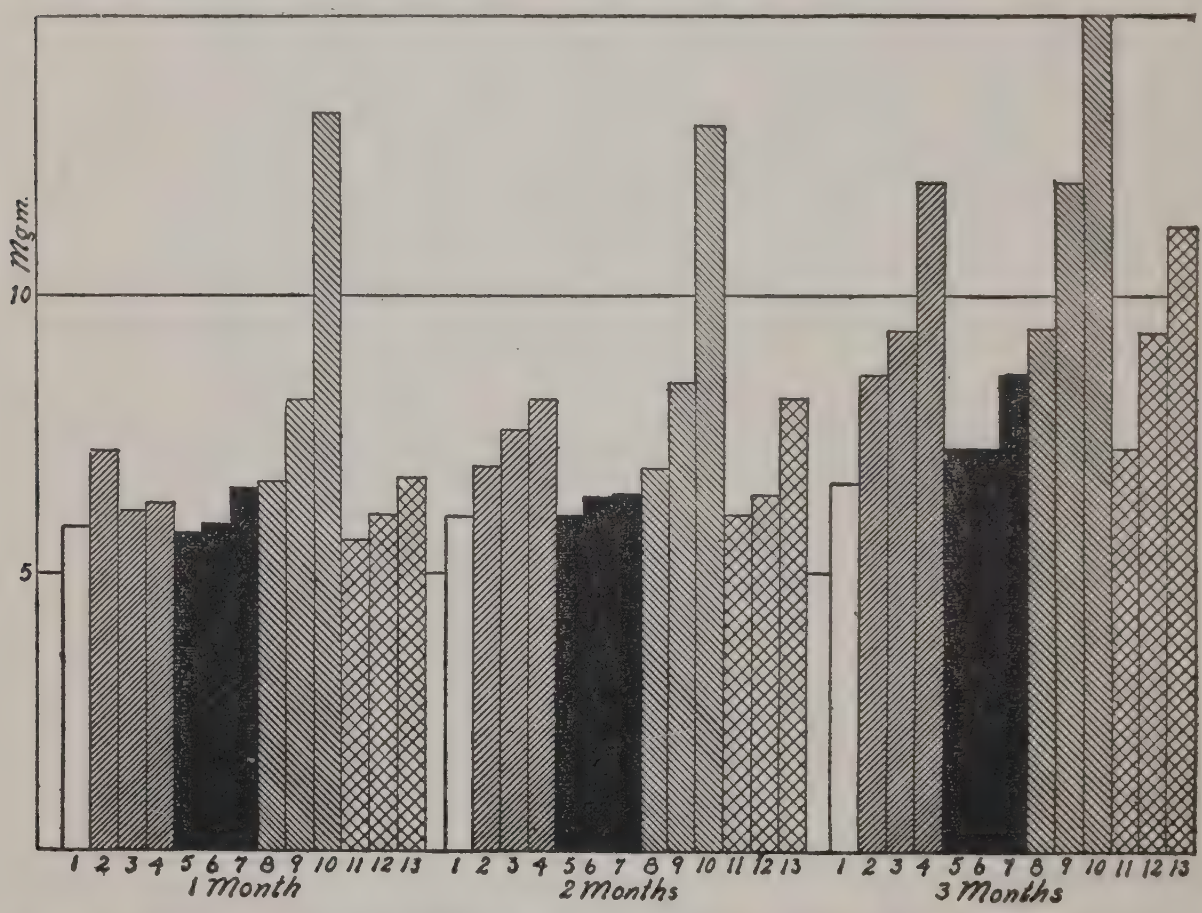

FIG. 10.—Diagram showing the influence of calcium carbonate, magnesium carbonate, limestone, and monocalcium phosphate on nitrate accumulation of Colby silt loam.

1=no treatment.
2=one-fourth calcium carbonate.
3=one-half calcium carbonate.
4=full calcium carbonate.
5=one-fourth limestone.
6=one-half limestone.
7=full limestone.
8=one-fourth magnesium carbonate.
9=one-half magnesium carbonate.
10=full magnesium carbonate.
11=0.1 per cent monocalcium phosphate.
12=0.1 per cent monocalcium phosphate+one-fourth calcium carbonate.
13=0.1 per cent monocalcium phosphate+full calcium carbonate.

The monocalcium phosphate apparently did not favor nitrification in the Colby soil, while in the sand this substance proved beneficial. A combination of the phosphate and calcium carbonate did not increase nitrification beyond that obtained from the use of calcium carbonate alone. The effect of these substances on the reproduction of soil organisms and on the formation of nitrates was similar. A review of the entire data is shown graphically in figure 10.

INFLUENCE OF CALCIUM CARBONATE, MAGNESIUM CARBONATE, LIMESTONE, AND MONOCALCIUM PHOSPHATE ON THE NITRIFICATION OF GELATIN IN SOIL

In order to study the effect of calcium carbonate, magnesium carbonate, limestone, and monocalcium phosphate on nitrification in Colby and in Plainfield sand to which nitrogenous material was added, a series of tests was made. After the soils had been treated with the forenamed substances separately and allowed to incubate for three months, 100-gm. samples were drawn and to each portion 23.6 mgm. of gelatin were added. The soil was placed in tumblers, which were covered with glass, and incubated for six weeks at 27° C. At the end of this time the nitrate content was determined. The results of this experiment are given in Table XII.

TABLE XII.—*Influence of calcium carbonate, magnesium carbonate, limestone, and monocalcium phosphate on the nitrification of gelatin in Colby silt loam and in Plainfield sand*

Treatment.	Nitrate nitrogen in 100 gm. of dry soil.			
	Colby silt loam.		Plainfield sand.	
	After 6 weeks.	Increase.	After 6 weeks.	Increase.
	Mgm.	*Mgm.*	*Mgm.*	*Mgm.*
None	20.87	....	9.06	....
One-fourth calcium carbonate	22.31	1.44	11.70	2.64
One-half calcium carbonate	25.00	4.13	10.57	1.51
Full calcium carbonate	28.43	7.56	11.12	2.06
One-fourth limestone	20.87	.00	11.70	2.64
One-half limestone	23.12	2.25	11.70	2.64
Full limestone	25.00	4.13	11.12	2.06
One-fourth magnesium carbonate	23.12	2.25	11.12	2.06
One-half magnesium carbonate	26.12	5.25	13.09	4.03
Full magnesium carbonate	25.00	4.13	12.24	3.18
0.1 per cent monocalcium phosphate	22.31	1.44	9.06	.00
0.1 per cent monocalcium phosphate+one-fourth calcium carbonate	23.12	2.25	13.09	4.03
0.1 per cent monocalcium phosphate+full calcium carbonate	28.43	7.56	12.24	3.18

In reviewing Table XII it will be seen that with the exception of one-fourth limestone treatment in Colby loam, and phosphate alone in Plainfield sand, there was an increase in nitrification in the treated soils. The increase in most cases was very slight, especially in the sand, where only about half of the nitrogen of gelatin apparently was nitrified, while in the Colby loam the greater part of the nitrogen of gelatin was nitrified. A comparison of the amount of nitrate formed in the soil to which magnesium or calcium carbonate was added, both with and without the addition of gelatin (Table XI), showed a relatively greater nitrate formation in the soils to which no organic nitrogen was added. These results were to be expected, since it is likely that some of the ammonia formed from the gelatin in the neutral or partly neutral soil escaped. On the other hand, the combined effects of the gelatin and the carbonates increased the multiplication of bacteria beyond that of the soil treated with gelatin alone. The great gain in the number of bacteria is no doubt

followed by an increase in assimilation of nitrates. These results agree with those obtained by Fred and Graul (*20*).

In the Colby silt loam the full calcium carbonate gave the best results while magnesium carbonate and limestone rank in the order named. The magnesium carbonate when added to the soil with gelatin did not give as good results as calcium carbonate. This difference may be due to the fact that the large increase in the number of bacteria caused by the magnesium carbonate treatment favored a greater assimilation of nitrate by the microorganisms. Plainfield sand apparently gave better results where the one-fourth or one-half neutralization was obtained. Very probably the sand releases more ammonia when neutral than does the Colby silt loam. In both soils the phosphate benefited nitrification but slightly.

INFLUENCE OF CALCIUM CARBONATE, MAGNESIUM CARBONATE, LIMESTONE, AND MONOCALCIUM PHOSPHATE ON NITROGEN FIXATION IN SOIL

An effort was made to study the influence which carbonate and phosphate treatments would have on the independent nitrogen-fixing organism, *Bacillus azotobacter chroococcum.* Three months after treatment, Colby and Plainfield soils were sampled and the soil placed in large soup plates, 1 per cent of mannit was added to each plate of soil, which was then inoculated with a culture of *B. azotobacter.* After incubating for four weeks in the greenhouse, the soils were dried and ground to a very fine powder in a ball mill. Total nitrogen determinations (Kjeldahl method modified to include nitrates) were then made. Duplicate determinations were made from each plate and an average of these taken. The results for this experiment are given in Table XIII.

TABLE XIII.—*Influence of calcium carbonate, magnesium carbonate, limestone, and monocalcium phosphate on nitrogen fixation in Colby silt loam and Plainfield sand treated with mannit and inoculated with Bacillus azotobacter*

Treatment.	Nitrogen in 100 gm. of dry soil.							
	Colby silt loam.				Plainfield sand.			
	After 4 weeks.		Average.	Increase.	After 4 weeks.		Average.	Increase.
	Mgm.	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*
None	275.0	275.0	275.0	....	63.0	61.0	62.0	....
One-fourth calcium carbonate	277.0	276.0	276.5	+1.5	59.0	59.0	59.0	−3.0
One-half calcium carbonate	273.0	275.0	274.0	−1.0	61.0	61.0	61.0	−1.0
Full calcium carbonate	276.0	276.0	276.0	+1.0	64.0	62.0	63.0	+1.0
One-fourth limestone	275.0	275.0	275.0	.0	61.0	59.0	60.0	−2.0
One-half limestone	273.0	275.0	274.0	−1.0	61.0	63.0	62.0	.0
Full limestone	278.0	279.0	278.5	+3.5	62.0	64.0	63.0	+1.0
One-fourth magnesium carbonate	277.0	276.0	276.5	+1.5	59.0	59.0	59.0	−3.0
One-half magnesium carbonate	278.0	276.0	277.0	+2.0	62.0	62.0	62.0	.0
Full magnesium carbonate	275.0	275.0	275.0	.0	63.0	63.0	63.0	+1.0
0.1 per cent monocalcium phosphate	274.0	276.0	275.0	.0	61.0	61.0	61.0	−1.0
0.1 per cent monocalcium phosphate + one-fourth calcium carbonate	279.0	282.0	280.5	+5.5	61.0	59.0	60.0	−2.0
0.1 per cent monocalcium phosphate + full calcium carbonate	283.0	277.0	280.0	+5.0	65.0	65.0	65.0	+3.0

From the data presented in Table XIII it appears that there was no decided gain in total nitrogen in the Plainfield sand, and only a small gain in Colby silt loam. It is difficult to explain why no gain, and in some cases a loss, of nitrogen was found. The loss may have been due to a disturbance in the balance of the flora of these soils. Since the mannit which was added to the soil, and the neutralization of some of the soil acids by the basic substances caused a great increase in the number of bacteria, it is possible that a part of the nitrate nitrogen was set free as elemental nitrogen. However, a gain in total nitrogen is shown in the Colby silt loam soil due to the carbonate and limestone treatment.

INFLUENCE OF CALCIUM CARBONATE, MAGNESIUM CARBONATE, AND LIMESTONE ON ORGANISMS IN PURE CULTURE

An effort was made to determine the influence of calcium carbonate, magnesium carbonate, and limestone on pure cultures of bacteria in sterilized soil. In order to simplify the work, only Colby soil was employed.

It was observed that when this soil is heated for a long period at a high temperature, there is a reduction in soil acidity. Other investigators have reported similar results in acid soils. Conner (*10*) showed a decrease in acidity when soil was heated to complete dryness. He suggested that possibly the acid silicates were hydrolyzed with the formation of a base. On heating soil to 140° C. for one hour, Sharp and Hoagland (*55*) noted a decrease in acidity. On the other hand, Schreiner and Lathrop (*54*) increased the acidity in an acid-reacting soil by heating the soil for three hours at 30 pounds' pressure, which in all probability changed the organic matter to such an extent as to increase the acidity. The work of Kelley and McGeorge (*30*) and Darbishire and Russell (*11*) showed a change in the soil consituents on heating, especially in the solid inorganic constituents, which were made more soluble.

In order to overcome as much as possible this reduction in acidity, various methods of sterilization were tried. Both dry and moist soils were sterilized in Erlenmeyer flasks, which were heated in steam under a pressure of 15 pounds for three hours. On testing this heated soil for its degree of acidity by the Truog zinc-sulphid test, the dry soil showed practically no change in its acid content, while the moist soil, one-half and full water-saturated, showed a considerable decrease. The very wet soil after sterilization contained less acid than that which received one half as much water. From the evidence it seems safe to conclude that the silicates of this soil are partially hydrolyzed with the formation of bases.

Further tests were conducted with dry soil. It was dried for 24 hours at about 45° C. and placed in flasks; these were then plugged and sterilized for three hours at 15 pounds' pressure. After sterilization, the soil received water sufficient to bring it to half saturation. This was done to determine if hydrolysis took place by adding water after the soil

had been sterilized. At the end of four days tests were made on the dry and on the watered soil. The acidity of both appeared unchanged.

From the results of the foregoing experiments it appears that dry Colby soil can be sterilized and sterile water added until the soil is half saturated, without interfering seriously with the original reaction of the soil. Therefore this method of sterilizing soil was adopted for all of the pure-culture work.

INFLUENCE OF CALCIUM CARBONATE, MAGNESIUM CARBONATE, AND LIMESTONE ON THE AMMONIFICATION OF BLOOD MEAL BY PURE CULTURES OF BACTERIA IN SOIL

COLBY SILT LOAM.—One-hundred-gm. portions of dry soil were each treated with calcium carbonate, with magnesium carbonate, and with limestone separately. To the soil of the entire series 2 per cent of dried-blood meal was applied. The treated soil was placed in 300-c. c. Erlenmeyer flasks and sterilized as previously stated. Sterilized water was then added to bring the soil to half saturation. The sterilized soil was then inoculated with water suspensions of *Bacillus tumescens* and *B. subtilis*. The flasks were incubated at 27° C. for seven days and at the end of this period the ammonia in each flask was determined. In Table XIV are recorded the complete data for this experiment.

TABLE XIV.—*Influence of calcium carbonate, magnesium carbonate, and limestone on the ammonification of dried blood with pure cultures in Colby silt loam*

Treatment.	Ammonia nitrogen in 100 gm. of dry soil.			
	Bacillus tumescens.		*Bacillus subtilis.*	
	After 7 days.	Increase.	After 7 days.	Increase.
	Mgm.	*Mgm.*	*Mgm.*	*Mgm.*
None	11.3	....	11.0	....
One-fourth calcium carbonate	15.5	4.2	....	....
One-half calcium carbonate	16.4	5.1	16.0	5.0
Full calcium carbonate	16.4	5.1	16.0	5.0
One-fourth limestone	11.2	−0.1	11.7	0.7
One-half limestone	14.6	3.3	13.0	2.0
Full limestone	14.8	3.5	13.9	2.9
One-fourth magnesium carbonate	15.7	4.4	12.5	1.5
One-half magnesium carbonate	16.5	5.2	17.6	6.6
Full magnesium carbonate	17.1	5.8	21.0	10.0

The data in Table XIV show clearly that the ammonification of dried blood by *Bacillus tumescens* or *B. subtilis* was increased when the sterilized soil was treated with calcium carbonate or with magnesium carbonate or with limestone. Magnesium carbonate gave better results than did calcium carbonate, while the latter gave better results than did the limestone. In every case full treatment with any of the compounds gave the largest increase of ammonia. This is in agreement with results obtained in unsterilized Colby silt loam two months after treatment with the limestone or calcium carbonate.

INFLUENCE OF CALCIUM CARBONATE, MAGNESIUM CARBONATE, AND LIMESTONE ON BACILLUS AZOTOBACTER IN STERILIZED SOIL

COLBY SILT LOAM.—Dry soil in 200-gm. portions was treated with calcium carbonate, with magnesium carbonate, and limestone, and placed in 500-c. c. Erlenmeyer flasks. To each flask was added 1 per cent of mannit by weight of dry soil. The flasks were plugged, sterilized, and sterile water was added to bring the soil to the proper moisture content. Inoculations of *Bacillus azotobacter* in pure culture were then made by adding a 1-c. c. suspension of the organisms to each flask. The entire set of flasks was incubated at 27° C., and after one, two, and three weeks plate counts, using mannit agar, were made. The results for this experiment are recorded in Table XV.

TABLE XV.—*Influence of calcium carbonate, magnesium carbonate, and limestone on Bacillus azotobacter in Colby silt loam treated with mannit*

Treatment.	Number of bacteria in 1 gm. of dry soil.		
	After 1 week.	After 2 weeks.	After 3 weeks.
None	<1,000	<1,000	<1,000
One-fourth calcium carbonate	6,602,000	4,170,000	2,440,000
One-half calcium carbonate	2,200,000	12,500,000	32,300,000
Full calcium carbonate	8,400,000	11,250,000	45,500,000
One-fourth limestone	<1,000	<1,000	<1,000
One-half limestone	<1,000	<1,000	<1,000
Full limestone	<1,000	850,000	26,000,000
One-fourth magnesium carbonate	26,200,000	3,620,000	10,350,000
One-half magnesium carbonate	5,150,000	47,000,000	268,000,000
Full magnesium carbonate	30,800,000	477,000,000	355,000,000

The results of this experiment showed the striking effect of magnesium carbonate on the multiplication of *Bacillus azotobacter* cells in Colby silt loam soil. Calcium carbonate caused an increase in the number of these organisms, but the increase was not as great as that obtained with magnesium carbonate. With limestone the increase was very small. This great gain with magnesium carbonate was obtained where amounts equal to full neutralization were applied. After the third week the one-half neutralization by magnesium carbonate also gave a large increase, while two weeks after treatment the greatest gain was shown.

INFLUENCE OF LARGE APPLICATIONS OF MAGNESIUM CARBONATE ON BACILLUS AZOTOBACTER IN STERILE SOIL

COLBY SILT LOAM.—Because magnesium carbonate when applied to give neutralization, increased the number of *Bacillus azotobacter* in sterilized Colby silt loam soil to a great extent, a further test was made to determine if heavier applications, enough added to give a distinct alkaline reaction, would continue to increase the reproduction of *Bacillus azotobacter*. These applications were made by adding magnesium carbonate sufficient to make 1¼, 1½, and double neutralization.

The data for this test, presented in Table XVI, showed that 1½ magnesium—carbonate treatment after one week gave a greater increase in the number of *Bacillus azotobacter* cells than was obtained by the 1¼ application; after two and three weeks, the 1¼ treatment gave a greater increase than was obtained by the 1½ treatment. In every case the heaviest application of magnesium carbonate caused less increase in the number of these organisms than did the lighter treatments. However, as compared with the control, the heavy application also gave a great increase.

In comparing the data of Table XVI with those of Table XV, it will be seen that 1¼ and in most cases 1½ magnesium-carbonate treatment caused a greater increase in the number of *Bacillus azotobacter* organisms in sterilized Colby silt loam than the full treatment. It is difficult to explain why such an enormous increase in the number of these organisms was obtained when more than enough magnesium carbonate was added to neutralize the soil acids. From the data in Table XVI, one is led to believe that this great multiplication in *Bacillus azotobacter* cells was due

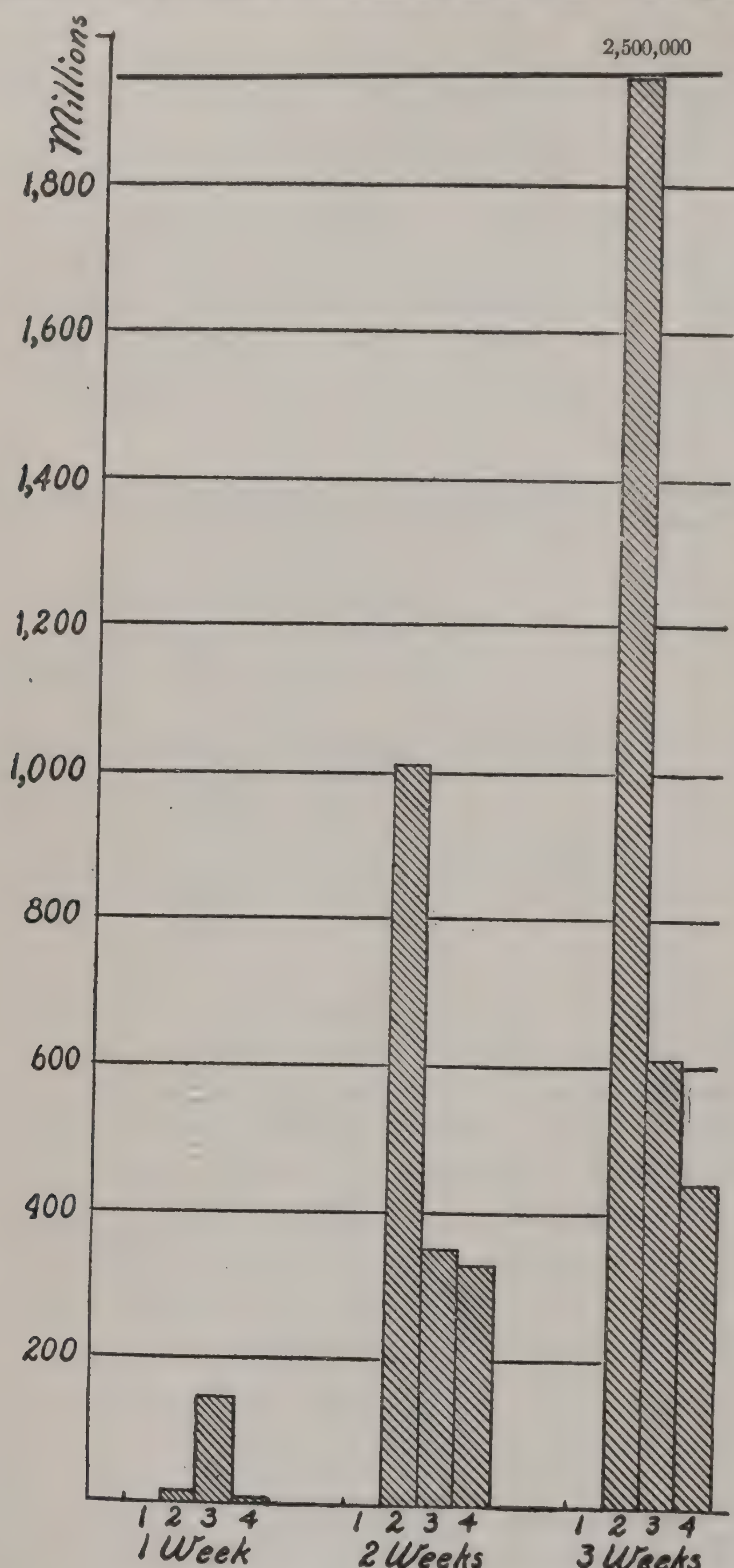

FIG. 11.—Diagram showing the influence of large applications of magnesium carbonate on *Bacillus azotobacter* in sterile Colby silt loam.

1=no treatment.
2=1¼ magnesium carbonate.
3=1½ magnesium carbonate.
4=double magnesium carbonate.

to something besides the correction of the soil acidity. The entire data in Table XVI are presented graphically in figure 11.

TABLE XVI.—*Influence of large applications of magnesium carbonate on Bacillus azotobacter in Colby silt loam treated with mannit*

Treatment.	Number of bacteria in 1 gm. of dry soil.		
	After 1 week.	After 2 weeks.	After 3 weeks.
None	<1,000	<1,000	<1,000
One and one-fourth magnesium carbonate	13,500,000	1,030,000,000	2,500,000,000
One and one-half magnesium carbonate	147,500,000	357,000,000	605,000,000
Double magnesium carbonate	10,300,000	338,000,000	481,000,000

INFLUENCE OF MAGNESIUM CARBONATE ON BACILLUS AZOTOBACTER IN STERILE NEUTRAL SOIL

MIAMI SILT LOAM.—Since magnesium carbonate increased the number of *Bacillus azotobacter* cells in an acid soil when more than enough of the carbonate was added to neutralize the acidity, an experiment was planned to determine the effect this compound would have on *Bacillus azotobacter* in a neutral soil. The soil selected for this work was Miami silt loam, previously described. Magnesium carbonate, along with 1 per cent mannit, was added in one-fourth, one-half, and full neutralization based on the acidity of Colby silt loam. The data for this test are tabulated in Table XVII.

TABLE XVII.—*Influence of magnesium carbonate on Bacillus azotobacter in sterile Miami silt loam treated with mannit*

Treatment.	Number of bacteria in 1 gm. of dry soil.					
	After 1 week.	Relative.	After 2 weeks	Relative.	After 3 weeks.	Relative.
None	3,020,000	100	37,500,000	100	51,000,000	100
One-fourth magnesium carbonate	2,870,000	95	52,500,000	140	127,000,000	249
One-half magnesium carbonate	44,000,000	1,456	11,200,000	30	137,000,000	269
Full magnesium carbonate	67,700,000	2,241	26,200,000	70	392,000,000	768

In studying the data in this table, it will be seen that in Miami silt loam, a neutral soil, where magnesium carbonate was applied in an amount great enough to neutralize all the active acidity in Colby soil, there was a great increase in the number of *Bacillus azotobacter* cells. At this time no explanation can be offered to account for the drop in number after two weeks where one-half and full magnesium carbonate were applied, since the other counts, made after one and three weeks with the one-half and full application gave a decided increase. The count made after the 3-week period showed the greatest increase. From the results of Table XVII it is clear that magnesium carbonate plays some rôle in stimulating the *B. azotobacter* organism other than that of neutralization.

INFLUENCE OF CALCIUM CARBONATE, MAGNESIUM CARBONATE, AND LIMESTONE ON BACILLUS RADICICOLA (ALFALFA) IN STERILE SOIL

COLBY SILT LOAM.—This experiment was planned to measure the effect of calcium carbonate, magnesium carbonate, and limestone on the reproduction of *Bacillus radicicola* in sterilized Colby silt loam soil. The alfalfa strain, which is supposed to be sensitive to acidity, was selected for this work. The soil was treated with 1 per cent of mannit, and the magnesium carbonate was added. Two-hundred-gm. portions of the treated soil were placed in 500-c.c. Erlenmeyer flasks. The flasks were then plugged and sterilized according to the method used in the preceding experiments. Inoculations were made by introducing a 1-c.c. suspension of the organisms.

From the data presented in Table XVIII it will be seen that calcium and magnesium carbonates increased greatly the number of alfalfa organisms in this soil. The magnesium carbonate after one week did not increase the number of these organisms to any greater extent than did calcium carbonate. The smaller amounts were surprisingly effective, though the increase was not as great as that obtained with larger amounts. Limestone increased the number of these organisms to a very slight extent.

TABLE XVIII.—*Influence of calcium carbonate, magnesium carbonate, and limestone on Bacillus radicicola (alfalfa) in sterile Colby silt loam treated with mannit*

Treatment.	Number of bacteria in 1 gm. of dry soil.		
	After 1 week.	After 2 weeks.	After 3 weeks.
None	12,500	1,200	1,200
One-fourth calcium carbonate	340,000,000	1,350,000,000	1,170,000,000
One-half calcium carbonate	1,310,000,000	1,650,000,000	1,600,000,000
Full calcium carbonate	1,750,000,000	1,620,000,000	1,870,000,000
One-fourth limestone	101,000	100,000	6,200
One-half limestone	227,000	5,000	50,000
Full limestone	2,450,000	4,170,000	240,000,000
One-fourth magnesium carbonate	87,000,000	1,212,000,000	1,540,000,000
One-half magnesium carbonate	4,100,000,000	1,112,000,000	1,770,000,000
Full magnesium carbonate	4,900,000,000	600,000,000	925,000,000

INFLUENCE OF CALCIUM AND MAGNESIUM CARBONATES ON BACILLUS RADICICOLA (LUPINE) IN STERILIZED SOIL

COLBY SILT LOAM.—Since the alfalfa strain of *Bacillus radicicola* was greatly benefited by the carbonates of magnesium and calcium, it seemed desirable to see what effect a similar treatment would have on the lupine strain of *B. radicicola*, which is frequently termed an acid-resistant organism. Limestone was eliminated in this experiment; otherwise the procedure for this test was similar to that in the preceding experiment. The results of this test are tabulated in Table XIX.

TABLE XIX.—*Influence of calcium carbonate and magnesium carbonate on Bacillus radicicola (lupine) in sterile Colby silt loam treated with mannit*

Treatment.	Number of bacteria in 1 gm. of dry soil.		
	After 1 week.	After 2 weeks.	After 3 weeks.
None	112,000	30,000	60,000
One-fourth calcium carbonate	875,000,000	3,770,000,000	5,530,000,000
One-half calcium carbonate	1,300,000,000	3,630,000,000	3,820,000,000
Full calcium carbonate	1,837,000,000	2,760,000,000	4,200,000,000
One-fourth magnesium carbonate	886,000,000	4,025,000,000	125,000,000
One-half magnesium carbonate	950,000,000	4,837,000,000	5,000,000,000
Full magnesium carbonate	1,750,000,000	8,850,000,000	9,170,000,000

From the data given in Table XIX it is evident that the lupine strain of *Bacillus radicicola* was benefited to as great an extent as the alfalfa strain when Colby soil was treated with magnesium or calcium carbonate. After the second and third weeks the counts showed that magnesium carbonate gave better results than did calcium carbonate. The untreated soil gave a slightly higher number than was obtained with the alfalfa strain under similar conditions. This difference may be due to the fact that the lupine strain is slightly tolerant to an acid reaction.

Here, as in the tests made with unsterilized soil, the smaller applications stimulated multiplication to a greater extent, in proportion to the amounts applied, than did the larger treatments. However, the maximum gain in number of bacteria was obtained with full magnesium-carbonate treatment. From the data obtained in this and the preceding experiment it is clearly evident that either magnesium or calcium carbonate (the magnesium to a somewhat greater degree than the calcium) greatly increased the number of legume bacteria, alfalfa and lupine strains, in an acid soil.

CONCLUSIONS

From a general review of the results of the experiments just described it is clearly shown that carbonates of calcium and magnesium when applied to acid Colby silt loam, acid Plainfield sand, and neutral Miami silt loam increase the number of bacteria. A greater increase was obtained in the acid soils than in the neutral Miami silt loam. These carbonates increased the number of bacteria in both sterilized and unsterilized soil. The sterilized soil was inoculated with an organism in pure culture. Not only was the number of bacteria in the soils increased by applications of magnesium and calcium carbonates, but an increase in activity of the various groups of bacteria was shown.

The form of carbonate which gave the best results was magnesium carbonate. There was an enormous increase in the number of bacteria produced by the magnesium carbonate over that of calcium carbonate. Ground dolomitic limestone did not prove quite as efficient as calcium

carbonate in increasing the number of soil bacteria. This variation can possibly be explained by the difference in solubility of these compounds. It was shown (*50*) that magnesium carbonate is more soluble in carbonated water than is calcium carbonate, and that calcium carbonate is more soluble than limestone. Dolomitic limestone, however, is less soluble than the nondolomitic. This order of solubility is in agreement with the order in which the bacteria of the soil responded to treatment with these different compounds.

It does not seem correct to say that the entire influence which these compounds had on the soil bacteria was due to the neutralization of the soil acids. When neutral soil was treated with magnesium carbonate or with calcium carbonate, the number of bacteria was increased, especially in the case of magnesium carbonate. Both in neutral and in acid soil, the latter doubly neutralized, magnesium carbonate increased the number of *Bacillus azotobacter*. All this would indicate that carbonates either serve in part as a stimulant or effect an indirect action on other compounds which are in turn rendered more soluble.

Since the magnesium and calcium carbonates increased the number of bacteria in acid soil when added in small amounts, and since application in acid soil of these compounds gave better results than when applied to neutral soil, it appears that the greater part of this influence on the bacteria was due to neutralization.

It may have been that the magnesium carbonate when added to the soil was partially converted into magnesium phosphate. Truog (*56*) pointed out the fact that for the amount of phosphate used, magnesium phosphate increased the phosphorus content of plants more than did other forms of phosphates. If this be the case, and the formation of magnesium phosphate takes place in soil when magnesium carbonate is applied, then magnesium chlorid and magnesium phosphate should give an increase in the number of bacteria. The results from the magnesium-chlorid treatment did not prove beneficial, however, even in so small amounts; the chlorin radical may have been toxic. Magnesium phosphate did not prove favorable to the reproduction of bacteria in acid soil but did in neutral soil. In the latter soil, the effect of magnesium phosphate was more beneficial than the effect of calcium phosphate. From this evidence it appears that magnesium phosphate in a soil favorable for the development of bacteria is a stimulant to the growth of bacteria. This action of magnesium phosphate on bacteria in soil may in part account for the enormous influence which magnesium carbonate exerts in increasing the number of bacteria in both neutral and acid soils.

From all the data obtained in the various experiments performed, magnesium carbonate appears to play an important part in the development of soil bacteria, much more so than does calcium carbonate. Magnesium phosphate, when applied to neutral soil, caused an increase

in the number of bacteria. In the neutral soil magnesium phosphate proved superior to calcium phosphate in stimulating the bacteria; in acid soil neither compound appeared to benefit the soil flora.

SUMMARY

(1) The number of bacteria in acid Colby silt loam and acid Plainfield sand is increased by the applications of calcium carbonate, magnesium carbonate, or limestone.

(2) Magnesium carbonate increases the number to a much greater extent than does either calcium carbonate or limestone.

(3) Monocalcium phosphate and dibasic magnesium phosphate slightly increase the total number of bacteria in neutral soil.

(4) Nitrification is benefited by limestone, calcium-carbonate, and magnesium-carbonate treatment. Magnesium carbonate in soil to which no nitrogenous substance was added favors nitrate accumulation more than does either calcium carbonate or limestone. The phosphates increase the accumulation of nitrate nitrogen to a very small extent. When gelatin was applied to the soil, magnesium carbonate did not benefit nitrification any more than calcium carbonate or limestone.

(5) Ammonification in Colby soil is benefited by all three forms of the carbonates, while in Plainfield sand a decrease in ammonia is shown. Monocalcium phosphate increases ammonification in both soils.

(6) Pure cultures of *Bacillus tumescens* and *B. subtilis* ammonify blood meal better when sterile Colby soil is treated with any one of the three forms of carbonates.

(7) A culture of *B. azotobacter* in the two soils treated with the carbonates and mannit fails to show an increase in total nitrogen in the sand and only a slight gain in the Colby soil.

(8) Pure cultures of *B. radicicola*, of both alfalfa and lupine strains, and *B. azotobacter* are greatly benefited when inoculated into sterile Colby soil previously treated with magnesium or calcium carbonate. Limestone barely increases the number of *B. azotobacter* in Colby soil. In neutral and acid soils treated with magnesium carbonate until the soils were strongly alkaline, *B. azotobacter* is greatly increased in munber over that of the untreated.

(9) From the data considered as a whole, magnesium carbonate is superior to calcium carbonate or limestone in stimulating the reproduction of bacteria in Colby silt loam and Plainfield soils. In general, the smaller applications of either compound give better results than do the heavier applications.

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HUMUS IN MULCHED BASINS, RELATION OF HUMUS CONTENT TO ORANGE PRODUCTION, AND EFFECT OF MULCHES ON ORANGE PRODUCTION

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INTRODUCTION

The formation of humus in a soil and its conservation are points that are usually given much weight in discussions of soil fertility. The free formation of humus in a soil from organic matter added to it is usually held to indicate that the soil its biologically active, and proper biological activity in a soil is conceded to be necessary under the usual agricultural practices. Hence, whatever the absolute value of soil humus may be as a factor in crop production, it is ordinarily conceded, other factors being equal, that a soil which readily forms humus when organic matter is added is superior to one that does not.

Previous work (*1*)[1] by this Office in southern California has shown that the mottling of Citrus leaves varied inversely with the humus content in the soil. In other work by the same writers (*2*) it was shown that on certain soil types in the general region of Riverside, California, the use of the mulched-basin system in Citrus groves improved the tree conditions more than the usual system of cultivation and furrow irrigation, and that different green-manure substances when used as a mulch affected tree growth and fruit setting in varying degrees.

It is therefore of practical value to obtain information about the rate of humus formation from various organic substances when employed either as a mulch or when worked into the soil, and to ascertain, if possible, whether the increase in humus is correlated with increase in tree growth and fruit setting.

This work reports the study of humus formation in mulched basins in Citrus groves, and the effect of different mulching materials on fruit production and tree growth. The work was done at Riverside.

The term "humus" as used in this paper is the brown-colored organic matter extracted from a soil by boiling it for two minutes in a 7.5 per cent sodium-hydrate solution, after the removal of calcium from the soil with 1 per cent hydrochloric acid.

The percentage of humus was determined colorimetrically by comparing the intensity of the color of the soil extract thus obtained with the intensity of a standard humus solution prepared from the humus ex-

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[1] Reference is made by number (italic) to "Literature cited," pp. 517-518

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tracted from peat. In the great number of humus determinations made by the Office of Biophysical Investigations in the Citrus areas studied in California not a single humus extract obtained has been off-color, when compared with the standard solutions prepared for comparison.

Gortner (4) obtained a black soil pigment from the soils he studied by extracting them successively with 4 per cent sodium hydroxid, 1 per cent hydrochloric acid, 4 per cent sodium hydroxid followed by water. The soil residue obtained from the last sodium-hydroxid extraction, when shaken with water in quantity, yielded the black pigment.

Soils from various Citrus areas studied in this work did not produce black pigment in appreciable amounts when they had been extracted with hydrochloric acid to the absence of calcium, boiled for two minutes in a 7.5 per cent sodium-hydroxid solution, and washed with hot dilute sodium-hydroxid solution on the filter till the "humus color" had been removed. It might be noted that a boiling 5 per cent solution of sodium hydroxid did not remove all the color from the soils, and that a 10 per cent solution removed no more than a 7.5 per cent solution.

Following Gortner's procedure, black pigments were obtained from some of the soils investigated in the Citrus areas of southern California.

It should be noted that the colorimetric reading should be made as soon as the humus extract is obtained, as the color partially fades out on standing.

As no evidence was obtained to indicate that any coloring matter was left in the soil after the extraction with boiling sodium hydroxid of the strength noted, and as the color tint was always the same as that of the standard used, it seems that this rapid method for humus determination is reliable for comparative studies, especially when used with soils in the same general area.

Another objection to the colorimetric method is that undecomposed organic matter, like dry alfalfa, sweet clover, etc., produces a color when treated in the manner just described for making humus determinations. This objection is also brought out by Gortner (4). This matter was looked into when the method was worked out. When dry hay susbtances were extracted with hydrochloric acid to the absence of calcium, the chlorin washed out, and the residue boiled for several minutes with a 7.5 per cent solution of sodium hydroxid, the organic materials yielded a yellow-colored solution entirely different in color from the brown to black humus color.

A number of readings of these organic extracts gave a density of color corresponding to about 0.0012 per cent humus in soil, if it is assumed that 1 per cent of the substances was to be added to the soil. This is about the working error in making humus determinations by the method used, as will be seen from Table II, and such errors could have no influence on comparative results in the kind of study here reported. Indeed there could never have been much of this off-color in the extracts, as a

uniform tint was always obtained from the soils studied in making humus extracts from them. The straw color produced by the extraction of soil with 1 to 2 per cent of undecomposed organic matter added was found to be so thoroughly obscured by the humus color of the soil that its presence did not interfere with the colorimetric reading.

It is well recognized that when hay material of many kinds is digested with hot hydrochloric acid and extracted with ammonia, a solution is obtained which in appearance and color can not be distinguished from a humus extract from a soil.

Much of the literature relating to humus is discussed by Schreiner and Shorey (*10*).

HUMUS FORMATION IN MULCHED BASINS

Experiment I.—Mulched basins, 15 feet in diameter, were installed in an orange grove near Riverside, Cal., in March, 1915. The soil is a red clay loam, derived from granite, which underlies the soil at a depth of 3 to 4 feet. One half of the basins were mulched with about 180 pounds of alfalfa (*Medicago sativa*) each and the other half with about 20 cubic feet of good cow manure each. One half of the basins in each mulching series received 200 pounds of lime dust each. This lime was a by-product from the flues of a neighboring cement plant. It was composed of about 45 per cent of calcium carbonate, about 20 per cent of calcium hydrate, about 10 per cent of calcium sulphate, about 1 per cent of potash, and a little phosphoric acid. The balance was mostly silicious material.

During August of the same year another row of orange trees in the same grove was basined and mulched with alfalfa. These basins were smaller than those just referred to, and less mulch was used. Part of these basins received about 100 pounds each of ground lime rock analyzing about 90 per cent calcium carbonate. The rest of the basins in the row were unlimed.

All surface organic matter was carefully removed, and soil samples were taken to a depth of 3 feet in these basins, usually from three basins in each experiment, the samples being separated into foot sections. Corresponding foot samples from the three basins were composited, and each composite sample was analyzed in duplicate. The percentage reported in the following tables are the averages to a depth of 3 feet. Usually the samples were collected just before irrigation.

Table I shows the average percentage of humus from time to time to a depth of 3 feet of soil in the basins under the various treatments during a period of from 12 to 17 months.

The low humus percentage is due in part to the removal of several inches of the surface soil in constructing the basins, which suggests incidentally that in making basins as little surface soil as possible should be removed.

TABLE I.—*Percentage of humus to a depth of 3 feet in mulched basins in orange groves on clay loam soil. Experiment I*

Date.	Basin treatment and percentage of humus.					
	Large basins installed in March.				Small basins installed in August.	
	Alfalfa alone.	Manure alone.	Alfalfa and lime.	Manure and lime.	Alfalfa alone.	Alfalfa and lime.
1915.						
Mar. 31	0.075	0.118	0.113	0.129	....	....
Aug. 13	.073	.130	.106	.112	....	....
Sept. 13	.075	.136	.147	.139	0.166	0.165
Oct. 12	.084	.153	.155	.160	.114	.092
Nov. 11	.098	.186	.185	.151	.118	.108
Nov. 20	.187	.188	.150	.151	....	....
Dec. 11	.198	.180	.188	.156	.121	.096
Average for 1915	.113	.156	.149	.143	.130	.115
1916.						
Mar. 17	.113	.201	.180	.179	.171	.115
Apr. 17	.111	.246	.287	.213	.257	.259
May 15	.076	.133	.181	.141	.133	.107
June 16	.112	.231	.288	.220	.165	.139
July 13	.081	.295	.272	.207	.117	.111
Aug. 7	.185	.292	.215	.227	.212	.175
Average for 1916	.113	.233	.237	.198	.176	.151
Ratio 1916 to 1915	.000	1.49	1.59	1.38	1.35	1.31

It will be observed that the determinations show a fluctuation in the percentage of humus from time to time. This is doubtless due partly to the difficulty of getting uniform soil samples. This factor, however, does not afford a complete explanation, because the same kind of fluctuation was noted when determinations were made on soils kept in pots in the laboratory, where better control conditions obtained and where leaching was avoided. Neither can the fluctuations be due entirely to the working error in making humus determinations, since these errors are of smaller magnitude than the variations in the humus content of the soils.

The following duplicate determinations made on March 31, 1915, the averages of which are given on the first line in Table I, illustrate the working error in making humus determinations by the method employed (Table II).

Heinze (*6*) states that after humus has been formed other bacteria, such as Azotobacter, commence to decompose it. McBeth (*9*) lays stress on the fact that the cellulose destroying organisms in the soil break down the organic matter in the soils with the formation of humus, and that the nitrifying organisms break the humus up into still simpler compounds through their nitrifying activities.

TABLE II.—*Working error in making humus determinations*

Soil No.	Humus.	Soil No.	Humus.	Soil No.	Humus.
	Per cent.		*Per cent.*		*Per cent.*
677	0.358	676	0.227	685	0.284
677	.358	676	.227	685	.284
678	.041	677	.051	686	.081
678	.039	677	.050	686	.081
679	.045	678	.023	687	.050
679	.046	678	.022	687	.050
670	.250	682	.192	688	.284
670	.250	682	.200	688	.284
671	.030	683	.056	689	.074
671	.028	683	.051	689	.070
672	.048	684	.022	690	.043
672	.046	684	.021	690	.042

From these considerations it might therefore be expected that the amount of humus in the soil in the basins would be subject to fluctuation, the amount present at any one time depending upon the ascendency of one or the other of these important groups of soil organisms.

The fluctuation in the percentage of humus makes it impracticable to attempt to determine the rate at which humus was formed between specific dates. It will be seen in Table I that there is frequently a decrease in the percentage of humus from one period to another, especially during the second year, a point that might possibly apply to determinations made by Gortner (5). He added silk, wool, flour, and alfalfa meal to soils, kept the materials in earthenware jars, and at the end of a year made humus determinations. In most instances he found less humus at the end of the year than he found in the treated soils when the experiment was started. It would have been of much interest if these determinations had been made frequently during the year. None of the losses of humus reported by him are greater than was sometimes found in a month's time in humification studies carried out under laboratory control in this Office, to be reported later.

A comparison of the amount of humus in the basins from year to year is made by averaging the percentage found during each of the years 1915 and 1916, as shown in Table I. At the bottom of the table will be found the ratio of the average percentage of humus in 1916 to that in 1915.

EXPERIMENT II.—In April, 1915, a block of orange trees in another grove on sandy loam soil was basined. The basins were about 12 feet in diameter, and the treatments were the same as in Experiment I, except that less lime was used.

The results of the humus determinations are given in Table III, which shows the fluctuation in the amount of humus from time to time. The ratio of the average percentage of humus in the basins in 1916 to that in 1915 is given in the bottom line in the table.

TABLE III.—*Percentage of humus to depth of 3 feet in mulched basins in orange groves on sandy loam soil. Experiment II*

Date.	Basin treatment and percentage of humus.			
	Alfalfa alone.	Manure alone.	Alfalfa and lime.	Manure and lime.
1915.				
May 3	0.169	0.159	0.214	0.196
Aug. 9	.128	.121	.126	.164
Sept. 4	.129	.146	.187	.189
Oct. 4	.230	.218	.283	.260
Oct. 30	.203	.209	.255	.307
Nov. 30	.185	.159	.181	.212
Average for 1915	.174	.169	.208	.221
1916.				
Mar. 17	.251	.241	.301	.286
Apr. 17	.227	.220	.296	.338
May 15	.129	.180	.163	.180
June 16	.216	.213	.309	....
July 13	.195	.167	.244	.248
Aug. 7	.175	.296	.243	.309
Average for 1916	.199	.220	.259	.272
Ratio, 1916 to 1915	1.14	1.30	1.24	1.23

EXPERIMENT III.—In October, 1915, a block of 60 trees in another orange grove of heavy sandy loam soil was basined and mulched. The basins were about 6 by 20 feet. The mulching materials used, the quantity of each, and the percentage of humus in the soil are given in Table IV. The percentages are averages to a depth of 3 feet. The amount of bean straw used was not enough to cover the soil, and so did not make an effective mulch. Some of these basins received in addition to the mulching material, 100 pounds ground lime rock, analyzing about 90 per cent calcium carbonate.

TABLE IV.—*Average percentage of humus to a depth of 3 feet in mulched basins in an orange grove on heavy sandy loam. Experiment III*

Basin treatment.	Percentage of humus.		Humus ratio—1916 to 1915.	Quantity of mulch and lime per basin.
	Oct. 13, 1915.	June 27, 1916.		
Alfalfa alone	0.223	0.316	1.41	125 pounds.
Alfalfa and lime	.160	.185	1.16	100 pounds of lime.
Bean straw alone	.243	.220	.91	70 pounds.
Bean straw and lime	.241	.240	1.00	100 pounds of lime.
Manure alone	.264	.226	.86	20 cubic feet.
Manure and lime	.244	.163	.67	100 pounds of lime.
Barley hay alone	.319	.247	.78	125 pounds.
Barley hay and lime	.235	.194	.83	100 pounds of lime.
Sweet clover alone	.254	.161	.63	125 pounds.
Sweet clover and lime	.237	.215	.91	100 pounds of lime.
Bur clover alone	.279	.203	.73	125 pounds.
Pine shavings alone	.266	.279	1.05	125 pounds.
Pine shavings and lime	.265	.206	.78	100 pounds of lime.

The basins with alfalfa as the mulch were the only ones which increased in percentage of humus during the interval given. Other experiments have shown that bean straw readily humifies. It is possible that if this had been applied in as large a quantity as the other substances, these basins would have increased in humus also. The lime was not consistent in its effect on the amount of humus formed.

It is to be noted, however, that the percentage of humus as found in the summer, after the application of the mulches in the preceding fall, does not necessarily indicate that in most of these basins the average amount of humus had actually decreased. As shown in Tables I and III, there are periodical fluctuations in the humus content, and the second set of determinations shown in Table IV may have been made at a time when the amount of humus was comparatively small. The figures in Table IV probably give an indication of the relative humifying activity in the basins. Each percentage given is an average of about six determinations.

Experiment IV.—A series of basins was installed in a lemon grove near Corona, Cal., in October, 1915. The soil is sandy loam in texture interspersed freely with gravel so that samples below 3 feet can not be taken with the ordinary soil tube.

The mulching materials used were alfalfa and manure and, in addition to the organic mulch, some of the basins received other artificial materials—viz, blood, phosphate, tankage, sulphur, bone meal, and lime. In addition to the basined rows, several control rows were retained, and were manured, irrigated, and cultivated in the usual way. The amount of manure applied to the trees in the control rows was the same as applied in the basins.

Soil samples were collected after the basins were made, on October 20, 1915, and another set was taken on June 29, 1916. The detailed humus determinations made on the latter date did not indicate that any of the artificial substances added to the mulches produced any definite influence on the humus content. The average percentage of humus to a depth of 3 feet in the alfalfa basins, manure basins, and in the control rows is given in Table V. Each percentage figure given in the table is made up of an average of about 21 determinations, each determination representing a composite of at least three samples.

Table V.—*Average percentage of humus to a depth of 3 feet in unmulched soil and in mulched basins in a lemon grove on light sandy loam soil. Experiment IV*

Soil treatment.	Percentage of humus.		Ratio 1916 to 1915.
	Oct. 20, 1915.	June 29, 1916.	
Basins with alfalfa mulch	0.360	0.306	0.85
Basins with manure mulch	.345	.361	1.05
Furrow irrigation and surface cultivation; manured	.551	.380	.60

The low initial percentage of humus in the basins is partly due to the fact that 5 or 6 inches of the surface soil were removed in making the basins, owing to sloping ground.

The percentage of humus in the manured basins in 1916 was about the same as in 1915, while in the alfalfa basins it was less. The decrease in the humus content in the manured soil furrow-irrigated and surface-cultivated is very appreciable, indicating that this system did not conserve the humus as well as the basin system.

The grove in which this experiment was conducted was deteriorating along with other groves in the neighborhood when the basins were installed. The whole grove was in better condition in October, 1916, but there was no apparent difference in the appearance of or the amount of fruit on the trees basined as compared with trees not basined. The mulch-basin system in experiments conducted by this office has always produced a quicker response on trees on clay loam soil than on trees on sandy loam or light sandy loam soil.

The deterioration of the grove above mentioned was evidently due to bad soil conditions, which quite likely affected the bacterial flora in such a way as to make it less efficient in converting the alfalfa into humus.

In most cases presented above the percentage increase in humus during a period of from 9 to 17 months was greater in the basins on the clay loam soil than in those on the sandy loam soil, with both manure and alfalfa as the mulch. On the whole, the manured basins gained slightly more in humus content on both types of soil than the alfalfa-mulched basins. No correction has been made for the amount of humus added with the manure. This material contained about 5 per cent of humus when applied, part of which undoubtedly found its way into the surface soil when the basins were irrigated. The percentage increase of humus in the manured basins therefore does not necessarily indicate that the manure had been humified to a greater extent than alfalfa. Data to be presented in another paper would indicate that the increase is likely due to the humus added with the manure.

In all cases where lime was added to manure in basins the increase in humus was somewhat less than when manure alone was used. In most cases the addition of lime to alfalfa in basins produced slightly more humus than when alfalfa was added alone. Frear and Hess (*3*) found that field plots receiving manure and lime contained less active humus than unmanured plots.

The amount of alfalfa or manure necessary to form a unit amount of humus can not be stated with exactness from the data available. In Experiments I and II the average increase in humus in the basins in 1916 over the average amount present in 1915 would indicate that 1 part of humus was formed from about 10 to 12 parts of alfalfa, and 1 part of humus from about 26 parts of manure. These calculations agree fairly

well with results stated by others (*7, p. 128–129*), but are necessarily only estimates.

The data obtained in the humus studies in mulched basins did not indicate that there was any very appreciable accumulation of humus in the lower soil depths due to leaching from the surface foot of soil. The detailed data showed that most of the change in humus content took place in the surface foot.

RELATION OF HUMUS CONTENT OF THE SOIL TO ORANGE PRODUCTION

EXPERIMENT I.—Picking records of the earlier basined trees in Experiment I were obtained from the company on whose grove the experiment was conducted, and are given in Table VI.

TABLE VI.—*Effect of different mulching materials on orange production. Experiment I. Picked May, 1917*

Basin treatment.	Average number of boxes of oranges per tree.			1917 yield corrected on basis of previous yields (boxes per tree).	Row No.	Tree numbers in row.
	1914	1915	1917			
Alfalfa alone	1.49	0.71	3.31	4.97	3	1 to 8
Alfalfa and lime	1.19	.58	3.61	6.69	3	26 to 33
Manure alone	1.78	1.00	2.16	2.56	3	9 to 15
Manure and lime	1.32	1.02	1.81	2.55	3	16 to 25

This appreciable difference in fruit production of trees mulched with alfalfa and manure, respectively, does not correlate with the humus content in the soil under the respective mulches, as may be seen from Table I. The manured basins average a slightly higher percentage of humus than the basins mulched with alfalfa.

It will also be noticed in Table VI that the trees basined and mulched with manure in March, 1915, had previously produced slightly more fruit than the trees mulched with alfalfa.

When the oranges were picked in the spring of 1917, the color of the oranges on the trees mulched with alfalfa was discinctly more golden than the color of the oranges on the trees mulched with manure.

EXPERIMENT II.—Individual tree picking records had not been kept of the trees in the grove in which this experiment was conducted. Individual tree picking records were obtained in the spring of 1917 from the company on whose grove the experiment was conducted. In this experiment a row of trees was left unbasined and was manured, furrow-irrigated, and cultivated in the usual manner. The amount of manure per tree was the same as that put into the basins.

The fruit production of the trees is given in Table VII. The number of trees used in each set of mulched trees was from 13 to 15.

TABLE VII.—*Influence on orange production of different mulches in basins and by furrow irrigation and cultivation. Experiment II. Picked June, 1917*

Soil treatment.	Average number of boxes per tree.	Average for each organic substance with and without lime.
Basins with alfalfa mulch alone	5.3	4.8
Basins with alfalfa mulch and lime	4.5	
Basins with manure mulch alone	4.3	4.0
Basins with manure mulch and lime	3.9	
Cultivated with manure alone, disked	2.9	2.5
Cultivated with manure and lime	2.3	

It is not known that the trees used in this experiment differed in yields previous to the installation of this experiment. However, as no individual tree picking records had been kept previous to the experiment, the comparative yields shown in Table VII do not carry the force they would if they could have been compared with the previous performance of the same trees. In comparing the fruit production in experiments I and III, it seems safe to infer that the results obtained in Experiment II were indicative of the effect of the different organic materials used.

It will be noticed in Table III that the percentage of humus in the manured basins was slightly higher than that in the basins mulched with alfalfa, a result agreeing with that obtained in Experiment I.

No appreciable difference in the color of the fruit from the trees differently treated was discernible.

EXPERIMENT III.—The block of 60 orange trees used in this experiment had been used in a study of individual tree performance by the Office of Horticultural and Pomological Investigations, Bureau of Plant Industry. Individual tree records for the previous six years had been obtained, and these were kindly furnished by the Riverside officials of that Office. The picking records of the 1916–1917 crop are given in Table VIII, together with the basin treatments. The basins were installed and mulched in October, 1915.

No effect of the lime on fruit production could be definitely determined; hence, the yields of all the trees mulched with the same organic matter have been averaged, including both limed and unlimed trees.

The alfalfa-mulched trees produced more fruit than the manure-mulched trees, which result agrees with the results obtained in Experiments I and II. It does not appear from Table VIII that any legume whatever is superior to a nonlegume or to manure. The basined trees mulched with sweet clover and bur clover did not produce as much fruit as those mulched with alfalfa, bean straw, manure, or barley hay.

The color of the oranges on the trees mulched with alfalfa and bean straw was distinctly more golden than the color of the oranges on the trees mulched with the other organic substances. The foliage on the bean-straw and alfalfa-mulched trees was considerably denser and greener than that on the other trees.

Table VIII.—*Effect of different mulching materials in basins on orange production. Experiment III. Picked May, 1917*

Basin treatment.	Yield of oranges corrected on basis of previous performance of trees.		
	Pounds per tree.	Oranges per tree.	Average weight per orange.
			Pounds.
Alfalfa hay	308	881	0.350
Bean straw	289	744	.389
Manure	261	611	.427
Barley hay	202	497	.407
Sweet clover hay	217	584	.372
Bur clover hay	216	530	.408
Pine shavings	163	402	.406

On comparing the results of the humus determinations shown in Table IV with the fruit production shown in Table VIII it is evident that the percentage of humus in the soil does not correlate with the fruit production. There is, however, a correlation between the fruit production and the ratio of the humus content in 1916 to that in 1915, but such correlation is not evident in Experiments I and II.

Closer examination of the picking records in Experiment III showed that the bean-mulched trees produced more first-class fruit of the more desirable sizes than the trees mulched with any of the other substances; the alfalfa-mulched trees were second in this respect; manure third; and barley fourth; while the trees mulched with sweet clover and pine shavings produced the least number of fruits of the first quality.

It may be that some toxic substance is formed in the decomposition of the pine shavings which might account for the poor condition of the trees and for the small yield obtained from them; also the shavings probably had a deleterious influence on nitrification. Redwood boxes for use in germinating Citrus seedlings proved unsuitable, because, when the root tips came in contact with the wood, they promptly died.

Oranges from the trees in Experiments I, II, and III were analyzed, the analyses being made by the branch office of the Citrus By-products Laboratory in Los Angeles. The analyses did not bring out any consistent differences in the fruits from trees receiving different fertilizer treatments.

There was a consistent difference in the specific gravity of the oranges from the limed trees and from the unlimed, but the difference was small. The average specific gravity of the oranges from the mulched and limed trees was 1.0012, and of those from the mulched trees, but not limed, was 1.0307. The specific gravity was calculated from the grading results, and not by individual determinations of separate fruits. The error in sizing by the grading machine would probably be compensated by the number of fruits included, as the entire crop was used in the calculation.

On the whole, the evidence obtained from these experiments does not show that the humus content of the soil correlates with orange production. Neither does the information obtained justify the statement that the humifying activity in the soil correlates with fruit production; but the results obtained would indicate that this point might be worthy of further study.

It appears from the results here presented that the most important function of organic matter as influencing orange production is not that of merely furnishing humus; that humus in itself is not the most important product of organic degradation for orange production. It appears from other work (*8*) that a more important function of organic matter is to make the plant food in the soil minerals more readily available. It was found that organic substances, especially in a freshly decomposing condition, dissolved plant food elements in appreciable amounts, even when the organic solvents contained practically no electrolytes. This function and that of promoting the biological activities of the soil seem to be more important rôles of organic matter in the soil than merely to furnish humus.

SUMMARY

This report presents a study on (*a*) the changes in humus content in soils in basins mulched with different organic substances, (*b*) the effect of lime on humus content in soils in mulched basins, (*c*) the relation of humus content in the soil to orange production.

By "humus" is meant the brown- to black-colored organic extract obtained from soil leached with 1 per cent hydrochloric acid to the absence of calcium and the soil residue boiled for two minutes in a 7.5 per cent sodium-hydrate solution.

Humus determinations in mulched basins in citrus groves showed a fluctuation in the percentage of humus from time to time.

The average percentage of humus increased more in basins on clay loam soil than in basins on lighter soil tubes, with manure and alfalfa as mulching materials.

Usually the percentage of humus in basins increased more when manure was used as mulch than when alfalfa was used as mulch. This seemed,

however, to be due more to the humus added with the manure, than to the greater "humification" of the manure over the alfalfa.

When manure alone was used as mulch in basins the increase in humus was greater than when lime was added with the manure.

In most cases when lime was added to alfalfa in basins greater increase in the humus content occurred than when alfalfa alone was used.

Blood, acid phosphate, bone meal, tankage, or sulphur did not show any appreciable influence on the changes of humus content in mulched basins.

It was not evident that there was any appreciable accumulation of humus in the lower depths of soil due to the leaching of humus from the surface foot of soil.

There was no evident correlation between the amount of humus in the soil in mulched basins and the amount of fruit on the trees.

There was no evident effect of lime on orange production in these experiments.

Alfalfa and bean-straw mulch in basins on the heavier soil types produced from 30 to 100 per cent more oranges per tree than manure mulch. Manure mulch produced more oranges per tree than either barley hay, sweet clover, bur clover, or pine shavings. These differences were obtained in the summer following the application of the mulches in the preceding fall.

Alfalfa mulch and manure mulch in basins on the lighter types of soil produced no observable differences on fruit production of lemons in the course of one year. This statement is based only on observation and not on picking records.

In all experiments so far conducted by this Office in the Riverside area, the mulched-basin system on the heavier soil types has produced favorable growth response in a few months. It usually takes longer to produce appreciable response on the lighter soil types.

It would appear directly from the work here reported, and indirectly from work elsewhere reported that the degradation products from freshly decomposing organic substances are more effective in orange production than the amount of "humus" formed. And the value of a given mulch does not necessarily depend upon its being a legume or nonlegume.

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RELATION OF KINDS AND VARIETIES OF GRAIN TO HESSIAN-FLY INJURY[1]

[PRELIMINARY REPORT]

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It has long been known that certain varieties of wheat (*Triticum* spp.) are injured less than others by the Hessian fly (*Mayetiola destructor*). Packard[2] mentions the Underhill, Mediterranean, Lancaster, and Clawson varieties as being noted for resistance. He states that the Underhill variety has been highly recommended for nearly a century.

Woodworth[3] made observations on 125 varieties of wheat grown at the California Experiment Station in 1886, 1887, and 1889, and noted the damage by Hessian fly in each. The Volo and Washington Glass varieties were not injured. Forelle; bearded wheat from Missoyen, Palestine; Polish; Bluegrass; Common March; Diamond; and Egyptian Imported were practically free from injury.

Roberts, Slingerland, and Stone,[4] in summarizing their observations on Hessian-fly injury in New York, conclude that

> the resisting power of varieties varies greatly

and that

> those with large, coarse, strong straw are less liable to injury than weak-strawed and slow-growing varieties.

Six varieties are mentioned which were not appreciably affected by the fly in 1901, although numerous other varieties in the same neighborhoods were much injured. These varieties were Dawson Golden Chaff, Prosperity, No. 8, Democrat, Red Russian, and White Chaff Mediterranean.

Gossard and Houser[5] made careful observations on 75 varieties of wheat and other grains grown at the Ohio Experiment Station in 1904, 1905, and 1906. They determined the percentage of stalks infested and of fallen straws. Their observations

> give but little support to the idea that there are immune varieties,

and they suggest that cases of supposed immunity may be explained by some other hypothesis. They state, however, that the most persistent

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[1] Contribution from the Entomological Laboratory (Paper No. 30) and the Department of Agronomy (Paper No. 13) cooperating. This paper embodies some of the results obtained in the prosecution of projects No. 8 and 67 of the Kansas Agricultural Experiment Station.

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search has never located a single Hessian-fly egg on oats, although eggs were found on many grasses.

Since 1906 the relation between varieties and injury from Hessian fly has received scant attention from investigators. Presumably the work of Gossard and Houser has been accepted as disproof of the claims of earlier observers that some varieties are resistant and others immune.

Recently claims of immunity put forth by growers of certain varieties, general observations by farmers in eastern and central Kansas that hard wheats are more susceptible to injury than soft varieties, and results of experiments at the Kansas Agricultural Experiment Station indicate that the subject is at least worthy of further investigation.

Experiments have been outlined to determine (1) the relative infestation and injury of different kinds, varieties, and strains of various small grain, and (2) why certain kinds and varieties are resistant or immune; or, if not, why they escape injury in some cases where others are badly injured. This paper is concerned primarily with the first problem.

EXPERIMENTAL DATA

The data reported in this paper were collected from 87 kinds and varieties of wheat (*Triticum spp.*), oats (*Avena sativa*), barley (*Hordeum spp.*), rye (*Secale cereale*), emmer (*Triticum dicoccum*), einkorn (*Triticum monococcum*), and spelt (*Triticum spelta*), planted in the Agronomy Nursery of the Kansas Agricultural Experiment Station in the fall of 1916. The different varieties were planted each in a row 50 feet long and 10 inches apart. Two plantings were made on each of two dates, September 15 and October 1. The soil was in excellent condition, moisture was plentiful, germination was prompt, and growth was normal in every respect. Hessian flies were numerous, and, as far as known, there was ample opportunity for all varieties to become equally infested. In this paper all varieties are tabulated in the order in which they were planted.

Eight of the varieties tested were from Australia (rows 1 to 8, inclusive, Table I), and had not been grown previously at Manhattan. The spring varieties and about half of the soft winter varieties had been obtained from various Experiment Stations in the United States in 1914 and had been grown at Manhattan for two years only before being included in this experiment. All of the hard winter varieties and about half of the soft winter varieties had been grown at Manhattan for several years and were thoroughly acclimated.

The relative number of eggs deposited on each variety was determined by taking five consecutive plants from the west end of each row and counting the total number of eggs on the leaves. The first count was made at the time of maximum deposition, September 25. Subsequent determinations were made for the early sown plots on October 2 and 7, and for the late sown plot on October 14. The total number of plants of each variety examined was 20.

The relative number of flaxseeds in each variety was determined by examining 50 consecutive plants of each row in each plot sown on September 15, and 25 consecutive plants of each row in each plot sown on October 1, making a total of 150 plants of each variety. All plants were taken from the western end of the plots, or, in other words, adjacent to those plants which were examined for eggs. The pertinent data for each variety are given in Table I.

That the Hessian fly is apparently able to discriminate between kinds and varieties of grain is shown by these data. For example, the total number of eggs per 100 plants in the early sown plots ranges from 40 for Culberson winter oats to 5,600 for Turkey winter wheat No. 2407. The proportion of plants on which eggs were laid varied from 20 per cent for Culberson winter oats and Michigan winter barley to 100 per cent for Tennessee winter barley and most of the varieties of wheat.

The close agreement in determinations made at different times, and the striking differences in the number of eggs laid on adjoining varieties indicate that the difference can scarcely be attributed to experimental error. Thus, Turkey winter wheat No. 2407, which showed the highest total number of eggs, also had the highest infestation on September 25, the second highest on October 10, when the second determination was made, and was among the highest on October 7, when the third determination was made. On the other hand, Culberson Winter oats, Michigan Winter barley, and einkorn had the lowest total infestation and the lowest on each date.

Row 12 (Polish wheat) had a total of 2,040 eggs per 100 plants, as compared with 280 for row 13 (einkorn). Row 15 (spring emmer) had a total of 660 eggs per 100 plants, as compared with 1,740 for row 16 (Black Winter emmer).

On the whole, the Hessian fly appears to have shown a preference for common wheat, as compared with barley, oats, einkorn, spring emmer, spelt, and durum wheat. Black Winter emmer, Poulard wheat, and Polish wheat were as heavily infested with eggs as many of the common wheats. Rye showed a very heavy infestation, the total number of eggs per 100 plants being 2,500, which is well above the average for all grains included in the test.

Varieties of the hard winter wheat class were more generally infested than soft winter wheat varieties. Thus, 27 varieties of hard winter wheat averaged 2,737 eggs per 100 plants, as compared with an average of 1,835 for 38 varieties of soft winter wheat. However, there are wide variations in each class. For example, No. 2408 and Mealy, which are soft, or semihard, varieties, were infested with 4,720 and 4,320 eggs, respectively, per 100 plants, showing almost as high an infestation as the most profusely infested varieties of hard wheat, and more than double that of some varieties. On the other hand, certain varieties of hard wheat, such as Defiance No. 2129, Red Winter No. 839, Improved Turkey No. 2382, and Pesterboden No. 205, had a lower infestation than the average of the soft, or semihard, varieties.

TABLE I.—*Relation between the kind and the variety of grain and the infestation of the Hessian fly*

Row No.	Variety of grain.			Grain planted on September 15.									Grain planted on October 1.		
	Kind.	Name.	Agronomy Department No.	Number of eggs per 100 plants.				Plants with eggs Sept. 25.	Average number of flaxseeds per 100 plants.	Plants with flaxseeds	Average number of eggs per infested plants Sept. 25.	Average number of flaxseeds per infested plant.	Number of eggs per 100 plants.	Number of flaxseeds per 100 plants.	Plants with flaxseeds.
				Sept. 25.	Oct. 2.	Oct. 7.	Total.								
								Per ct.		*Per ct.*					*Per ct.*
1	Common white wheat	King's Early	1,401	1,800	480	40	2,320	100	183	35	18.0	3.5	900	10	12
2	.....do	Bunyip	1,402	1,860	360	0	2,220	100	123	39	18.6	3.7	100	72	24
3	White spring wheat	Bobs	1,403	1,100	280	20	1,400	60	114	42	10.8	3.1	380	36	10
4	Durum wheat	Huguenot	1,404	320	60	0	380	100	34	15	3.2	2.2	100	8	6
5	.....do	Cleveland	1,405	160	160	200	520	60	118	34	2.6	3.0	40	10	6
6	Hard red wheat	Cedar	1,406	2,020	240	20	2,280	100	68	39	20.2	1.7	100	12	10
7	Durum wheat	Indian Runner	1,407	180	660	20	860	40	28	16	2.5	1.8	20	14	8
8	Common white wheat	Tuscany Solid Straw	1,408	620	1,260	100	1,980	100	157	36	6.2	4.4	580	......	......
9	Durum wheat	Kubanka	298	420	0	0	420	60	35	13	7.0	2.8	20	20	14
10	Hard red spring wheat	Marquis	297	740	180	80	1,000	100	71	32	7.4	2.5	440	......	......
11	Poulard wheat	......	......	1,980	40	20	2,040	100	35	11	19.8	3.8	280	6	4
12	Polish wheat	......	......	1,920	40	80	2,040	80	46	20	24.0	2.4	0	4	4
13	Einkorn	......	......	220	40	20	280	60	0	0	3.6	0	0	0	0
14	Spelt	......	......	460	120	0	580	80	55	26	5.7	1.9	380	2	2
15	Spring emmer	......	......	460	200	0	660	80	0	0	5.7	0	100	0	0
16	Winter emmer	Black Winter	......	1,500	140	100	1,740	80	144	50	18.7	3.4	280	34	32
17[a]	Winter oats	Winter Turf	5,165	......	......	......	......	......	......	......	......	......	......	......	......
18	.....do	Culberson Winter	5,158	40	0	0	40	20	0	0	1.0	0	0	0	0
19	Winter barley	Tennessee Winter	8,042	220	0	0	220	100	1	1	2.2	1.0	0	0	0
20	.....do	Michigan Winter	7,096	120	40	0	160	20	13	5	6.0	1.5	0	0	0
21	Rye	Rye	......	2,400	120	0	2,520	100	0	0	24.0	0	60	0	0
22	Hard red winter wheat	Kanred	2,401	1,960	160	20	2,140	100	140	42	19.6	4.3	160	36	22
23	.....do	Crimean Select	2,402	4,560	640	100	5,300	100	124	37	45.6	3.2	120	22	14

24	.....do	Turkey	2,407	4,820	680	100	5,600	100	258	57	48.2	3.2	460	68	34
25	Soft red winter wheat	German	2,403	1,240	220	80	1,540	100	71	30	12.4	2.4	100	38	20
26	Hard red winter wheat	Malakof	2,071	2,640	260	180	3,080	100	172	51	26.4	3.4	140	18	16
27	Soft red winter wheat	Hybrid	2,408	4,020	560	140	4,720	100	55	25	40.2	2.6	160	32	20
28	Hard red winter wheat	Kharkof	382	2,480	120	100	2,700	100	280	60	24.8	4.8	180	76	36
29	.....do	Ghirka	385	1,840	400	100	2,340	100	157	41	18.4	4.3	120	22	14
30	.....do	Turkey	570	4,380	460	40	4,880	100	101	51	43.8	3.8	100	36	26
31	.....do	Red Winter	839	1,340	200	160	1,700	100	158	50	13.4	3.5	220	46	26
32	Soft red winter wheat	Mealy	69	3,860	380	80	4,320	100	165	47	38.6	5.0	20	14	12
33	Hard red winter wheat	Malakof	113	3,460	200	40	3,700	100	219	48	34.6	6.6	140	42	52
34	.....do	Turkey Hybrid	196	1,320	280	140	1,740	100	289	65	13.2	5.4	140	50	29
35	.....do	Pesterboden	205	1,240	200	120	1,560	80	203	45	15.5	4.2	160	38	20
36	.....do	Beardless Hard Winter.	1,210	2,280	200	20	2,500	100	60	28	22.8	2.3	180	8	8
37	.....do	Kharkof	1,443	1,540	160	100	1,800	100	121	45	15.4	3.5	60	30	18
38	.....do	Turkey	2,039	2,820	440	20	3,280	100	151	46	28.2	3.5	20	70	34
39	.....do	.....do	2,042	2,120	120	20	2,260	100	173	57	21.2	3.8	60	54	30
40	.....do		2,059	1,540	220	20	1,780	100	171	45	15.4	3.4	120	30	10
41	.....do	Bearded Fife	2,094	1,620	460	20	2,100	100	185	58	16.2	3.1	220	80	36
42	.....do	Red Winter	2,101	1,280	520	60	1,860	100	285	62	12.8	5.0	160	8	6
43	.....do	Alberta Red	2,105	1,980	200	100	2,280	100	228	55	19.8	4.7	200	56	32
44	.....do	Defiance	2,123	2,260	360	20	2,640	100	268	58	22.6	5.6	220	54	30
45	.....do	.....do	2,129	1,200	160	0	1,360	100	158	41	12.0	4.1	60	20	16
46	.....do	Red Winter	2,132	2,560	320	60	2,940	100	30	9	25.6	2.8	140	4	2
47	.....do	Champanka	2,136	2,480	200	80	2,760	100	141	42	24.8	3.5	120	34	28
48	Soft red winter wheat	Red Hussar	64	480	440	220	1,140	100	93	47	4.8	1.8	200	52	16
49	Hard red winter wheat	Hungarian	65	1,380	480	100	1,960	100	103	45	13.8	2.3	340	36	18
50	Soft red winter wheat	Nigger	73	1,860	300	140	2,300	80	17	6	23.2	3.0	80	6	6
51	.....do	Fulcaster	83	1,380	420	100	1,900	100	19	10	13.8	2.5	220	6	2
52	.....do	Beechwood Hybrid	100	1,060	300	360	1,720	100	3	3	10.6	1.0	0	2	2
53	.....do	Miracle	106	1,400	280	40	1,720	100	12	4	14.0	0	200	8	6
54	.....do	Marvelous	408	2,900	40	240	3,180	100	59	24	29.0	2.6	260	40	22
55	Hard red winter wheat	Washington Hybrid	194	540	480	80	1,100	80	73	27	6.7	2.5	200	0	0
56	.....do	Binkel	2,353	1,380	260	100	1,740	100	161	59	13.8	2.3	120	22	18
57	Soft white wheat	Michigan Bronze Selection.	2,365	1,380	60	120	1,560	100	15	10	13.8	2.0	140	0	0
58	Hard red winter wheat	Improved Turkey	2,382	800	520	80	1,400	100	201	54	8.0	4.0	140	32	24

[a] Did not germinate.

TABLE I.—*Relation between the kind and the variety of grain and the infestation of the Hessian fly*—Continued

Row No.	Variety of grain.			Grain planted on September 15.									Grain planted on October 1.		
	Kind.	Name.	Agronomy Department No.	Number of eggs per 100 plants.				Plants with eggs Sept. 25.	Average number of flaxseeds per 100 plants.	Plants with flaxseeds	Average number of eggs per infested plants Sept. 25.	Average number of flaxseeds per infested plant.	Number of eggs per 100 plants.	Number of flaxseeds per 100 plants.	Plants with flaxseeds.
				Sept. 25.	Oct. 2.	Oct. 7.	Total.								
								Per ct.		Per ct.					Per ct.
59	Soft red winter wheat	Bucanera	2, 404	980	80	40	1, 100	100	246	54	9. 8	5. 0	160	46	32
60	do	North Allerton	2, 405	1, 660	140	40	1, 840	100	150	47	16. 6	2. 5	160	46	28
61	do	Currell Select	2, 406	1, 580	480	120	2, 180	100	3	2	15. 8	2. 0	100	8	4
62	do	Harvest Queen	9	860	280	60	1, 200	100	42	13	8. 6	10. 5	280	8	6
63	do	do	19	1, 740	140	20	1, 900	100	44	11	17. 4	2. 7	60	4	4
64	do	Alabama Bluestem	33	1, 440	160	80	1, 680	100	184	52	14. 4	3. 7	140	36	20
65	Soft white wheat	Hybrid	59	780	140	80	1, 000	100	24	10	7. 8	2. 1	60	4	2
66	do	do	66	1, 700	20	80	1, 800	100	46	17	17. 0	1. 7	80	6	4
67	Soft red wheat	Valley	70	500	220	40	760	80	8	7	6. 2	1. 1	100	0	0
68	Soft red winter wheat	Rudy	77	960	160	120	1, 240	100	6	4	9. 6	1. 6	60	2	2
69	Soft white winter wheat	Dawson Golden Chaff	78	2, 600	420	80	3, 100	100	3	3	26. 0	0	80	0	0
70	Soft red winter wheat	Dietz	84	1, 320	320	60	1, 700	100	15	7	13. 2	1. 7	120	0	0
71	do	Currell Prolific	90	1, 360	140	40	1, 540	100	38	14	13. 6	2. 0	140	2	2
72	do	Bearded Purple Straw.	109	1, 080	160	60	1, 300	100	23	10	10. 8	2. 6	1, 120	0	0
73	do	Gipsy	111	1, 820	20	80	1, 920	100	157	32	18. 2	4. 1	80	14	6
74	do	Jersey Fultz	116	880	200	80	1, 160	100	71	23	8. 8	3. 0	280	0	0
75	Soft white winter wheat	Hybrid	198	1, 320	160	80	1, 560	100	87	34	13. 2	1. 7	300	6	6
76	do	do	201	1, 860	40	0	1, 900	100	90	36	18. 6	3. 0	40	16	12
77	do	do	203	1, 980	200	60	2, 240	100	52	22	19. 8	2. 3	60	2	2
78	White club wheat	Washington Hybrid	204	1, 480	160	60	1, 700	100	53	19	14. 8	2. 6	160	0	0
79	Soft red winter wheat	Illini Chief [a]	223	1, 820	200	80	2, 100	80	0	0	22. 7	0	260	0	0
80	do	Zimmerman	2, 084	860	240	100	1, 200	100	207	65	8. 6	2. 9	140	24	18

81	.....do	Sibley New Golden	2, 088	1, 300	140	220	1, 660	100	202	57	13. 0	2. 7	600	36	16
82	.....do	Fultz	2, 156	1, 680	120	160	1, 960	100	132	44	16. 8	2. 7	20	4	4
83	.....do	Currell	2, 157	840	180	20	1, 040	100	65	23	8. 4	3. 1	40	4	4
84	.....do	German Emperor	2, 161	1, 320	160	240	1, 720	100	298	61	13. 2	6. 6	80	30	20
85	.....do	Deihl Mediterranean	2, 169	1, 780	180	80	2, 040	100	48	12	17. 8	4. 5	80	0	0
86	.....do	.....do	2, 175	1, 520	220	140	1, 880	100	40	16	15. 2	3. 1	20	14	10
87	.....do	Poole	2, 180	580	20	180	780	100	133	47	5. 8	3. 2	40	58	28

[a] Illini Chief is also known as Finley and Early Carlyle.

Perhaps the most significant result is the high mortality of the eggs and larvæ, and as a result the relatively low infestation with flaxseed of all varieties and especially certain ones. The figures are open to criticism, so far as the absolute mortality is concerned, since more eggs may have been laid on the ends of the rows, where the egg counts were made, than on the adjoining plants, which were used for the flaxseed determinations. However, this criticism would not hold for the relative mortality for the different varieties.

As will be seen, no flaxseeds were found in einkorn, spring emmer, Culberson Winter oats, rye, and Illini Chief wheat. Very low infestations, 5 per cent or less, were recorded for Tennessee Winter barley and for Beechwood Hybrid, Currell Selection, and Dawson Golden Chaff wheats. The data for rye and for Illini Chief and Dawson Golden Chaff wheats are especially significant, in view of the fact that 2,000 or more eggs per 100 plants were laid on each.

Of the hard wheats, Red Winter No. 2132 is especially worthy of mention, since it had only 30 flaxseeds per 100 plants, as compared with over 200 for other varieties of the same class. Only 9 per cent of the plants of this variety were infested with flaxseeds, as compared with a range of from 28 to 62 per cent for other varieties of this class.

In general, the data show a low, or no infestation, with flaxseeds for rye, barley, oats, durum wheat, Poulard wheat, Polish wheat, spelt, emmer, and einkorn. The average number of flaxseeds per 100 plants for 38 varieties of soft winter wheat was 76.1, as compared with 173.7 for 27 varieties of hard winter wheat, an increase for the latter of more than 225 per cent.

These conclusions are based on the rows planted on September 15, but essentially similar results were secured from those rows planted on October 1. The infestation on these plots was much lighter than in the former case, and the data are less conclusive. It will be noticed, however, that those varieties which were not infested or which show a low infestation in the first plantings exhibit a similar characteristic in the latter.

Field tests.—In 1915 a bushel of Illini Chief wheat was secured by the Entomology Department of the Kansas State Agricultural College and planted in four localities in the State where the Hessian fly was abundant.

The Illini Chief was practically free from injury in all cases. At Manhattan it showed less than a 1 per cent infestation, while Turkey wheat in an adjoining plot was infested practically 100 per cent.

An examination of the plants in the fall and spring indicated that the fly showed no preference for either variety, the eggs being equally numerous on both plots. In the Illini Chief the maggots were able to work their way down to the crown of the plant, but at this point development appeared to be arrested and the larvæ died.

At Winfield, Kans., the Illini Chief was sown in the center of a 40-acre field of hard wheat. Determinations in the spring showed that 95 per cent of the plants of hard wheat were infested, as compared with about 10 per cent for the Illini Chief.

In 1916, a plot of Illini Chief wheat grown by the side of Turkey wheat showed an infestation of from 3 to 5 per cent, as compared with 95 to 100 per cent for the Turkey variety.

While these tests appear to show that Illini Chief is somewhat resistant to the Hessian fly, it should not be assumed that it is the best variety to grow. In Kansas it is one of the least hardy of all winter-wheat varieties and will survive none but the mildest winters.

CONCLUSIONS

The Hessian fly is able to discriminate between different kinds and varieties of grain. Eggs were laid on all the kinds and varieties of grain studied, but very sparingly on winter oats, winter barley, einkorn, spring emmer, spelt, and durum spring wheat.

On the average, fewer eggs were laid on soft winter wheat than on hard red winter wheat, but exceptions in both cases were found.

There appeared to be a large mortality of eggs or larvæ on all kinds and varieties studied. This appeared to be greatest for rye, einkorn, spring emmer, winter oats, and Illini Chief wheat. Very few flaxseeds were found on winter barley, and on Beechwood Hybrid, Currell Selection, and Dawson Golden Chaff wheats.

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WILT DISEASES OF OKRA AND THE VERTICILLIUM-WILT PROBLEM

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INTRODUCTION

The investigation of the okra-wilt disease thus far reported has led to some confusion as to the cause of this malady. Atkinson (*3*)[2] mentions the fact that okra (*Abelmoschus esculentus*) is sometimes attacked by a disease similar to the wilt of cotton (*Gossypium herbaceum*). He states that a fungus for which he proposes the name "*Fusarium vasinfectum*" is invariably found in the vascular system of cotton and okra affected with this disease. Smith (*23*), in a more extensive study of the wilt diseases of cotton, watermelon (*Citrullus vulgaris*), and cowpea (*Vigna sinensis*), found an ascigerous fungus associated with the diseased plants. He regarded this new genus as the perfect form of *Fusarium vasinfectum* Atkinson, and renamed the latter fungus "*Neocosmospora vasinfecta*." Smith notes the occurrence of a wilt disease of okra, and regarding *N. vasinfecta* remarks (*p. 31*):

> It probably occurs also on okra, although the identification is not complete, depending solely on the character of the symptoms, on the presence of similar macroconidea and microconidia, and on the occurrence of the disease in the same localities, no cultures or cross inoculations of the okra fungus having been made and no perithecial fruits having been discovered.

Doubts as to the genetic relationship of *Neocosmospora vasinfecta* and *Fusarium vasinfectum* are expressed by Higgins (*12*), Butler (*5*), and Wollenweber (*31*, *32*). It appears from the inoculation and pure-culture work of the latter that *N. vasinfecta* Smith is to be regarded as a saprophyte entirely distinct from the vascular parasite *F. vasinfectum* Atk.

Clinton (*8*, *p. 268*) notes the occurrence of a wilt disease of okra in Connecticut which he considers as the same as that previously reported by Atkinson. The wilt of okra as it occurs in North Carolina is described

[1] Since the completion of these studies, the writer has been transferred to the position of Pathologist, Hawaiian Agricultural Experiment Station.

[2] Reference is made by number (italic) to "Literature cited," p. 544–546.

by Stevens and Wilson (*25*), with an additional statement of the disease by Wilson (*29*). They attributed this disease to *Fusarium vasinfectum.*

No inoculations of okra plants with *F. vasinfectum* are recorded by Atkinson, Clinton, Smith, or Stevens and Wilson. A species of Fusarium was found constantly associated with the wilt of okra, and the disease was regarded as the same as the cotton-wilt. Orton (*16*) states that in his experience okra has contracted the disease when planted in fields affected with cotton-wilt.

Wollenweber (*31*, *32*) reports a wilt disease of okra similar to that previously attributed to *Fusarium vasinfectum;* but *Verticillium albo-atrum* Reinke and Berthold, instead of the Fusariumu fungus, was constantly present in the vascular ducts. Successful inoculations were secured with *V. albo-atrum*, and the fungus was recovered from the wilting plants.

It would appear that there are two similar wilt diseases of this crop induced by two quite different vascular parasites. It was for the purpose of testing this latter hypothesis that the investigation herein recorded was undertaken. While okra as a crop is restricted in general to the home garden and is relatively of little importance, a study of the wilt diseases of this plant is of general significance to the whole problem of wilt disease.

THE GENERA VERTICILLIUM AND ACROSTALAGMUS IN RELATION TO WILT DISEASES

Verticillium albo-atrum was first described by Reinke and Berthold (*22*) in 1879 as the cause of a wilt disease of potato (*Solanum tuberosum*) in Germany. This appears to be the earliest record of species of Verticillium associated with wilt disease. The work of Reinke and Berthold was generally overlooked until the investigation of the leafroll (*Blattrollkrankheit*) and similar diseases of *S. tuberosum* brought it again to the attention of pathologists. Recently the presence of *V. albo-atrum* in wilting potato plants has been noted by several investigators: Appel (*2*), Spieckermann (*24*), Pethybridge (*18*), (*19*), Störmer (*26*), Wollenweber and Schlumberger (*34*), Wollenweber (*30*, *31*, *32*), Orton (*17*). Wollenweber (*31*, *32*) reports this organism as the cause of a wilt disease of a *S. tuberosum*, *S. melongena*, and *Abelmoschus* (*Hibiscus*) *esculentus*. A similar disease of snapdragon (*Antirrhinum* sp.) was described by Brown (*4*) and the causal fungus determined by the present writer as *V. albo-atrum*. *V. dahliae*, a species closely related to *V. albo-atrum*, is described by Klebahn (*14*) as the cause of a wilting of dahlias. Aderhold (*1*) also mentions a species of Verticillium in connection with thrombosis of currants (*Ribes* spp.—"*Johannisbeere*") and gooseberry (*Ribes* spp.—"*Stachelbeere*").

The causal organisms of a series of diseases of several host plants variously described as wilt disease, thrombosis, bluestem, etc., have been relegated by the respective authors to the genus Acrostalagmus. Owing

to the confusion existing in the genera Verticillium and Acrostalagmus, a discussion of the interrelations of these form groups is necessary at this time.

Among those writers who have obtained and described Acrostalagmus-like fungi may be cited the following: Van Hook (*27*) discusses a wilt disease of ginseng (*Panax quinquefolium*) which he found associated with a vascular-inhabiting fungus. It is said to closely resemble *Acrostalagmus albus* Preuss. Rankin (*20*) renamed the ginseng-wilt fungus "*Acrostalagmus panax*" Rankin, without giving a technical description of the fungus or stating reasons for the change. Whetzel and Rosenbaum (*28*), in mentioning this disease of ginseng, attribute it to a species of Acrostalagmus. Guéguen (*10*) describes a new species of Acrostalagmus causing a wilt disease of the China aster Reine-Marguerite (*Aster* sp.), and to this fungus he gave the name *A. vilmorinii.* A variety of this organism he later mentions (*11*) as associated with a disease of fruits of the cacao (*Theobroma cacao*). Lawrence (*15*) attributes a new disease of black raspberry (*Rubus occidentalis*)—that is, the bluestem disease—to a species of Acrostalagmus, for which he proposes the name "*Acrostalagmus caulophagus*." Rankin (*21*), in a preliminary report of a thrombotic disease of maple trees (*Acer* spp.), mentions that a species of Acrostalagmus is associated with the trouble.

Corda (*9, p. 15*) established the genus Acrostalagmus to accommodate an organism, *A. cinnabarinus*, which was said to differ from Verticillium by forming its spores in heads at the tips of the conidiophores. Hoffman (*13*) has rightly explained the complicated structure of the sterigma as represented by Corda, and likewise the collection in heads of the singly abscissed conidia. The conidia are held together by virtue of an hygroscopic slime, which takes up water in the presence of a sufficiently moist atmosphere, forming a sphere of water at the tip of each sterigma. In these water droplets the conidia are observed to float freely. With excess moisture the drops rupture and the conidia slip down the conidiophore. However, in the absence of moisture the conidia cling tenaciously to the tips of the sterigma in the form of irregular rounded masses of varying sizes. If such material is placed under a dry cover slip without being allowed to come in contact with the latter, and examined microscopically while a small water drop is brought into the vicinity of the dry spore head, the spore aggregate will be seen gradually to round up and the conidia to float more or less actively within the spherule (Pl. 17, B,D).

In the genera Acrostalagmus and Verticillium we now have the following contradictory and imperfect characterization and limitation of forms. In Verticillium the singly abscissed and characteristically singly borne conidia may adhere in the presence of moisture, forming terminal heads—that is, water droplets containing conidia. In Acrostalagmus the conidia, characteristically united in heads, soon separate if the humidity of the environment is in excess of the maximum for the moisture drops

to retain their form, leaving but one immature conidium on the sterigma tip, as in Verticillium.

Furthermore, in the genus Verticillium, characterized by singly borne or readily separating conidia, we have the anomalous condition of a section of the genus set aside for those species in which the conidia are held together by slime—that is, section Gliocephalum [1] Saccardo (1886). This section of the genus accommodates forms in which the conidia are united in slimy heads—that is, Acrostalagmus.

The fact that the work of Reinke and Berthold (*22*) was so generally overlooked is responsible for the confusion of the genera Verticillium and Acrostalagmus. These investigators studied *A. cinnabarinus*, on which Corda established his genus Acrostalagmus, and concluded that this form genus must be united with the older genus Verticillium Nees. They changed the name of Corda's fungus to *Verticillium cinnabarinum*. In view of the above, there seems to be no doubt that the genus Acrostalagmus Corda should be united with the genus Verticillium Nees. Klebahn (*14*) supports this view.

The species of Acrostalagmus, described as causing a vascular disease of ginseng, China aster, and black raspberry, may prove upon further work to be identical with each other and with *V. albo-atrum*, as proved in this paper, for the strains of the latter fungus isolated from okra, cotton, eggplant (*Solanum melongena*), and potato. Culturally, at least, the strains of the species of Acrostalagmus from ginseng and raspberry, in so far as they have been studied by the writer, are not to be distinguished from *Verticillium albo-atrum*. The organisms *A. vilmorinii* Guéguen and *V. dahliae* Klebahn, as described by their authors, hardly differ sufficiently from *V. albo-atrum* to be given specific rank. Possibly this is the better treatment of such related forms until their identity is established by careful cross-inoculation work with pure cultures. The minor cultural differences found by the writer in cultures of *V. albo-atrum* from different hosts are present in parallel cultures of the same strain. Strains of *V. albo-atrum* which are morphogically indistinguishable, isolated from different hosts, and capable of producing the same symptoms of disease by cross-inoculation, seemingly should be considered identical.

The available data on the several strains of Verticillium and Acrostalagmus thus far described as plant parasites are brought together in tabular form (Table I), in order that comparison may be readily made. The hypothetical identity of all of these strains, suggested by the similarity of this data, is strengthened if one consults and compares the descriptions by the several authors. The existing differences seem to be those of variety rather than of species.

[1] Saccardo's section Gliocephalum of the genus Verticillium (SACCARDO, P. A. SYLLOGE FUNGORUM. v. 4, p. 159. Patavii, 1886) is given as Gliocladium by Engler and Prantl (1900) apparently through error. (ENGLER, A., and PRANTL, K. NATURLICHEN PFLANZENFAMILIEN. Teil 1, Abt. 1**, p. 418, 432. Leipzig, 1900.)

TABLE I.—*Data of several species and strains of Verticillium and Acrostalagmus arranged for comparison*

Organism.	Conidia.		Sclerotia.		Sclerotial cells.		Length of conidiophore branches.
	Limits of size.	Average size.	Limits of size.	Average size.	Limits of size.	Average size.	
	μ.	μ.	μ.	μ.	μ.	μ.	μ.
V. albo - atrum strain 1717 from okra.	5.1 to 11 by 2.1 to 3.0.	6.8 by 2.4...	21 to 91	34 by 44	7 to 10	8.4 by 8.7	13.5 to 22.0
V. albo - atrum strain 2784 from potato.	4.2 to 9.3 by 1.7 to 4.2.	6.4 by 2.4...	31 to 70	45 by 54	5 to 10	8.2 by 8.2	....
V. albo - atrum strain 1685 from eggplant.	5.1 to 8.5 by 2.1 to 4.2.	6.5 by 2.6...	31 to 87	51 by 51	....	7.3 by 9.4	....
V. albo - atrum strain 2985 from snapdragon.	....	....	28 to 70	39 by 57	3 to 15	....	15.0 to 38.0
V. albo - atrum by Reinke and Berthold.	5.0 to 12.0 by 2.0 to 3.5.	....	....	....	....	....	....
V. albo-atrum as given by Klebahn.	4.0 to 6.0 by 1.5 to 2.5.	....	....	54	....	....	12.0 to 45.0
V. dahliae by Klebahn.	4.0 to 7.0 by 1.5 to 2.0.	....	....	50	5 to 6	....	16.0 to 27.0
A. albus by Van Hook.	2.0 to 5.0 by 1.0 to 2.0.	....	....	....	....	....	10.0 to 25.0
A. caulophagus by Lawrence.	3.0 to 7.0 by 2.0 to 3.0.	....	....	....	7 to 12	....	....
A. vilmorinii by Guéguen from China aster.	5.0 to 7.0 by 2.5 to 3.0.	....	20 to 70	50 to 60	....	....	....
A. vilmorinii from cacao.	6.0 to 7.0 by 3.0 to 4.0.	....	....	None.	....	....	....

For the reasons above set forth, it is evident that the genus Acrostalagmus must be united with the older genus Verticillium. In view of this condition, the following forms, if not identical with *V. albo-atrum*, must at least be regarded as belonging to the genus Verticillium: *A. albus* from ginseng-wilt disease, described by Van Hook (*27*) and renamed *A. panax* by Rankin (*20*); *A. vilmorinii* Guégen, from the China aster and the fruits of the cacao; and *A. caulophagus* Lawrence, the cause of the bluestem disease of black raspberry. Of the remaining 12 imperfectly characterized and apparently saprophytic species of the genus Acrostalagmus some are no doubt identical with species of Verticillium, and are just as incompletely delineated. The ubiquitous *V. lateritium* Berk., commonly present on decaying potatoes, is possibly the same as *V. innabarinum* (Corda) Reinke and Berthold.

FUSARIUM VASINFECTUM AND VERTICILLIUM ALBO-ATRUM, CAUSES OF THE WILT DISEASES OF OKRA

FUSARIUM VASINFECTUM

The species of Fusarium from okra- and cotton-wilt considered in this work are regarded as identical with each other and with the species of Fusarium isolated from "frenching" cotton by Atkinson (*3*) and named by him "*F. vasinfectum.*" The cross-inoculation work herein recorded proves the casual relation of *F. vasinfectum* to the wilt disease of okra,

and establishes the pathologic identity of the cotton- and okra-wilt strains beyond reasonable doubt.

Certain strains of the species of Fusarium causing cotton-wilt were observed by Wollenweber (*31*, *32*) to produce an aromatic odor (lilac) when cultured on starchy media such as rice; other strains less commonly isolated lacked this property. These latter strains were designated "*F. vasinfectum* var. *inodoratum*" by Wollenweber. As a further indication of the identity of the species of Fusarium on okra with the species of Fusarium on cotton, it should be noted that both the odor-forming and the non-odor-forming strains have been isolated several times from okra. While the ability to generate this odor is of doubtful specific value, since other species of the section Elegans (*Wollenweber, 32*) possess this property, and this ability has been observed to be lost in culture (*Carpenter 7, p. 206*), the fact that the species of Fusarium from okra and cotton agree in this character is significant.

Normal [1] cultures of this species of Fusarium develop in 1 to 3 weeks at room temperature (Pl. A). The best results are secured with plant stems, potato cylinders, and other vegetable media. Morphologically *F. vasinfectum* is scarcely distinguished from the other vascular parasites of the section Elegans of this genus.

Potato-cylinder cultures (Pl. A) develop an ocherous-salmon-colored pionnotes with 3- to 5-septate conidia (Pl. 17, L-M). Blue-gray sclerotia, similar to those of *F. oxysporum* Schlecht. (7, *Pl. A*, *1*), are generally present on this medium. The slight violet color of the upper part of the pionnotes as represented in Plate A, 3, has never been seen in other closely related species and is possibly of differential value. Stems of *Melilotus alba* and *Gossypium* sp. are useful in developing the sporodochia, which are likewise of an ocherous-salmon color. The plate of *F. oxysporum* in an earlier paper (7, *Pl. A*, *2*, 5) illustrates stem cultures of *F. vasinfectum* equally well. On steamed-rice medium a more or less brilliant red color soon appears, later becoming tinged with various shades of purple and blue, especially in subdued light. Normal conidia are not usually present in rice cultures, the value of this medium being the color reaction and the formation of an aromatic substance with an odor suggesting lilac (7, *p. 206*). Chlamydospores (Pl. 17, I) are formed in large numbers on this medium.

The following measurements show the size and percentage of the variously septate, normal, conidia (Pl. 17, L, M) found in strains of *F. vasinfectum* from okra and cotton:

F. vasinfectum, strain 2709, isolated from okra. Culture, 27-day-old stem of *Melilotus alba*, without pionnotes. Normal triseptate conidia, 60 per cent. Limits of size: 25.5 to 40.8 by 4.2 to 5.1 μ. Largest normal triseptate conidium, 40.8 by 5.1 μ.; smallest, 25.5 by 4.2 μ. Average

[1] For a discussion of the idea "normal" and other special terms as used in relation to species of Fusarium, see Wollenweber (*33, p. 255–257*).

size of 10 normal triseptate conidia, 32.5 by 4.6 μ. One-septate conidia, 20 per cent. Abnormal conidia and microconidia, 20 per cent.

Normal conidia on rice culture 2½ months old: Triseptate, 23.8 to 30.0 by 4.0 to 5.0 μ. Five-septate rare, 56.0 by 3.6 μ.

F. vasinfectum strain 3203 from okra. Pionnotes on a 25-day-old stem culture of *Melilotus alba*. Limits of size of 28 normal triseptate conidia: 23.8 to 34.0 by 3.8 to 4.2 μ. Average size, 28.4 by 3.9 μ. Largest triseptate conidium, 34.0 by 4.2 μ. Smallest triseptate conidium, 23.8 by 3.8 μ.

F. vasinfectum strain 3242 from okra. Normal triseptate conidia, 34.0 to 41.0 by 3.4 to 5.0 μ. Four-septate conidium 40.8 by 4.2 μ.

F. vasinfectum var. *inodoratum* strain 3257 from okra. Thirty-four-day-old *Melilotus* stem culture with pionnotes. Normal triseptate conidia 60 per cent. Limits of 17 spores, 32.3 to 45.9 by 3.4 to 4.8 μ. Average, 37.4 by 4.2μ. Four-septate, 35 per cent. Limits of 13 spores, 34.0 to 44.2 by 4.2 to 4.8 μ. Average, 39.0 by 4.2 μ. Five-septate, 5 per cent. Limits of four spores, 37.4 to 45.9 by 4.2 to 4.9 μ., average four spores 42.0 by 4.4 μ.

F. vasinfectum var. *inodoratum* strain 3258 from okra. Thirty-four-day-old pionnotes culture. Normal triseptate conidia 76 per cent. Limits of 9 spores, 27.2 to 41.6 by 3.4 to 4.6 μ. Average, 34.0 by 4.2 μ. Four-septate conidia, 19 per cent. Limits of 13 spores, 35.7 to 42.5 by 3.4 to 5.0 μ. Average, 37.8 by 4.1 μ. Five-septate, 5 per cent. Limits of 6 spores, 34.0 to 45.9 by 3.8 to 4.7 μ. Average, 40.0 by 4.2 μ.

Parallel cultures of *F. vasinfectum*, strain 1855, from cotton and strain 3592 from okra were prepared upon cotton stems as a medium. At the age of 45 days, 50 normal triseptate conidia from a pionnotes of each culture were measured to obtain the relative sizes. The maximum, minimum, and average length and width of conidia in each culture are shown in Table II. There is a close correspondence of the measurements of conidia from the two strains, considering that these measurements were made from but one culture of each strain.

TABLE II.—*Comparative size of normal triseptate conidia of Fusarium vasinfectum from okra and cotton*

	F. vasinfectum 1855 from cotton.	*F. vasinfectum* 3592 from okra.
	μ.	μ.
Maximum length	37.4	44.2
Minimum length	27.2	27.2
Maximum width	5.1	5.1
Minimum width	3.4	3.4
Average length	37.0	35.5
Average width	4.2	4.0

Fusarium vasinfectum Atkinson.

Sporodochia and perfect pionnotes present, in mass ocherous-salmon colored, the conidia being of the Elegans type, 3- to 5 septate, sickle-shaped, constricted at the apex and pedicillate at the base (Pl. 17, L,M). Conidiophores verticillately branched. Normal triseptate conidia present up to 100 per cent, 23.8 to 46.0 by 3.4 to 5.1 μ. Four-septate conidia up to 35 per cent, 34.0 to 44.0 by 3.4 to 5.0 μ. Five-septate conidia up to 5 per cent, 34.0 to 56.0 by 3.6 to 5.0 μ. Microconidia may be present in subnormal cultures up to 100 per cent, 4.0 to 14.0 by 2.0 to 3.5 μ. in size. Chlamydospores (Pl. 17, I) ellipsoidal to round, terminal, intercalary and conidial; when unicellular measuring from 8 to 15 μ. Blue-gray sclerotia on potato cylinders. Strong lilac odor on rice and other starchy media. Vascular parasite, cause of a wilt disease of *Gossypium herbaceum*, *G. barbadense*, and *Abelmoschus esculentus*.

VERTICILLIUM ALBO-ATRUM

Verticillium albo-atrum Reinke and Berthold is classified by Engler and Prantl in the section Eu-Verticillium of the genus Verticillium of the Mucedinaceae-Hyalosporae-Verticillieae. The conidophores (Pl. 17, A–D) are verticillately branched, conidia which readily fall being formed at the tips of all the branches. The distinction between the three sections of the genus—that is, Eu-Verticillium Sacc., Oncocladium Wallr., and Gliocephalum Sacc. is not sharply drawn. In the latter the conidia are held together by slime, while in Eu-Verticillium and Oncocladium this is not the case. In the opinion of the writer, *V. albo-atrum* should more appropriately be placed in the section Gliocephalum, for this is where it naturally belongs, if its characters are determined on the substratum (Pl. 17, B, D). If examined in water mounts, rarely more than one conidium would be found on each sterigma tip (Pl. 17, C), and the fungus would erroneously be placed with the section Eu-Verticillium.

DESCRIPTION OF THE FUNGUS

The conidia are ellipsoidal (Pl. 17, A), unicellular, 4.0 to 11.0 by 1.7 to 4.2 μ, abscissed singly from the tips of verticillate-branched conidiophores. They may or may not cling to the tips of the sterigma in rounded masses. In the absence of sufficient moisture in the air, relatively dry, rounded aggregates of spores accumulate; but with more moisture present spherical drops appear on the sterigma tips by virtue of the hygroscopic slime in which the conidia are embedded (Pl. 17, B). With additional moisture the drops rupture, leaving one immature conidium clinging to the sterigma. These masses measure from 3 μ to a size where the water drop breaks. The verticillate branches of the conidiophores are 1 to 7 to a whorl or virtel, more commonly 3 to 5, and these in turn may bear secondary branches in virtels. The branches are from 13 to 38 μ long, disposed in virtels 30 to 38 μ apart along the conidiophore. Conidiophores consisting of a terminal branch and two primary virtels, are about 100 to 120 μ in length, while those bearing four primary virtels measure 250 to 300 μ. Conidiophores with 7 to 8 primary virtels are occasionally

seen in petri-dish cultures. The terminal branch is usually 1 to 3 times longer than the virtel branches and measures from 15 to 60 μ.

The mycelium is hyalin at first, becoming brownish with age. It is septate, 2 to 4 μ in diameter, but is often swollen, all gradations from slightly swollen threads to large, thick-walled, and knotted sclerotia, and chlamydospore-like cells being present (Pl. 17, G). The sclerotium is at first but swollen, closely septate mycelium, which enlarges and knots itself into a variety of forms. The measurements of sclerotia of strains 1685, 1717, 2784, and 2985, recorded in Table I, were made only from the more or less rounded aggregations. Such measurements of irregular formations varying so greatly in size are only of general significance. Certain strains of *V. albo-atrum* cultivated by the writer produce sclerotia abundantly in a few days in petri-dish cultures. Macroscopically, their presence is manifested by a beautiful black zonation of the colonies, as illustrated in Plate 19. In other strains, and sometimes in other cultures of the same strain, these sclerotial rings either do not develop at all or only after a long time; yet these forms produce abundant sclerotia if cultivated on potato cylinders and other vegetable media. The entire growth then frequently consists of a black confluent layer of sclerotia and hyphæ. Parallel cultures of the same strain differ sufficiently with respect to the characters of the conidiophores and sclerotia formation so that specific determinations based on slight differences of these characters and unsupported by inoculation tests are of doubtful value.

Verticillium albo-atrum Reinke and Berthold.

Conidia ellipsoidal, unicellular, 4.0 to 11.0 by 1.7 to 4.2 μ, abscissed singly from the sterigma tips of verticillate conidiophores. Primary whorls or virtels of branches, 1 to 8 in number, 30 to 90 μ apart, sometimes bearing secondary virtels. Branches 1 to 7, usually 3 to 5 in number, 13 to 38 μ long, tapering, straight to slightly bowed. Conidiophores 100 to 300 μ or more in length. The terminal branch of the conidiophore is from 15 to 60 μ long. Conidia may or may not collect in heads on the sterigma tips. Mycelium septate, hyalin to brown with age; may become swollen into chlamydospore-like chains of closely septate, knotted masses. These aggregates constitute the sclerotia of this fungus. Vascular parasite, cause of a wilt disease of okra, potato, eggplant, cotton, snapdragon, and probably of species of Abutilon and Xanthium, ginseng, black raspberry, China aster, and dahlia. *V. albo-atrum* may prove to be the cause of the Verticillium-wilt disease reported on currants and gooseberries by Aderhold (*1*).

OCCURRENCE OF FUSARIUM VASINFECTUM AND VERTICILLIUM ALBO-ATRUM IN WILT DISEASES OF OKRA

Verticillium albo-atrum was found constantly inhabiting the vascular system of wilt-diseased okra plants (Pl. 27) in New Jersey, where this crop is of considerable importance. This organism was also isolated from similar material from Monetta, S. C., Birmingham, Ala., Middle River, Cal., and Medford, Oreg., specimens from Middle River and Medford having been collected by Dr. Wollenweber.

On the other hand, a Fusarium indistinguishable from the cotton-wilt Fusarium was constantly obtained from wilting okra collected at

Florence, Sumter, and Charleston, S. C., and Wrightsboro, N. C. According to Clinton (*8*), the Fusarium-wilt of okra occurs in Connecticut. *Fusarium vasinfectum* was not obtained by the writer from okra-wilt in New Jersey; and *Verticillium albo-atrum* was isolated from this host in the South only in the few mentioned localities.

V. albo-atrum was isolated from a wilt disease of the weeds *Abutilon* sp. and *Xanthium* sp. in New Jersey; and from spontaneous wilt of cotton plants in rows adjoining the experimental plot at Arlington, Va.

The wilt diseases of the several plants brought about by *F. vasinfectum* and *V. albo-atrum* manifest the same symptoms, so that the real cause of the trouble is safely to be determined only by cultural means. There is a lack of turgor in the leaves first in evidence in those parts farthest removed from the veins (Pl. 20–23). The lower leaves are first affected, wilt, and drop off one or two at a time. Frequently the plant does not die for a long time, but continues a dwarfed existence. This is especially true of the Verticillium-wilt. If the plants are cut longitudinally and crosswise, it will be seen that the vascular tissue is brown or black (Pl. 18), the discloration being traceable from the small roots to the top of the stem and into the petioles. Microscopic examination of thin sections of this material shows that the vessels are plugged with the mycelium of the parasite, which interferes with the conduction of moisture to the aerial portions of the plant (Pl. 17, E). The host tissues do not appear to be invaded or broken down, the vascular inhabitant merely living as a saprophyte on the fluids of the vessels, and injuring the host plant only by mechanical obstruction of the latter. Whether there are injurious products secreted in the metabolism of the fungus detrimental to the plant in other ways is yet to be demonstrated. The parasitism of these fungi is, then, a mechanical interference with the nutrition of the host and not our usual conception of this term.

METHOD OF TESTING PARASITISM

In order to demonstrate the ability of *V. albo-atrum* and *F. vasinfectum* to produce wilt diseases of okra and to gain a knowledge of the relation of these organisms to other host plants, approximately 1,000 inoculations [1] and cross-inoculations with pure cultures were made. Strains of *V. albo-atrum*, isolated from okra, eggplant, potato, and snapdragon, were used to inoculate okra; strains from okra, snapdragon, and eggplant were used to inoculate eggplant; and the strain from okra was used to inoculate cotton. Similarly okra plants were inoculated with *F. vasinfectum* isolated from okra and cotton; and cotton was inoculated with this fungus isolated from cotton and okra.

The general method used in the inoculations may be summarized as follows: Selected okra seed, or seed of other plants to be used, were disinfected in a solution of formalin, rinsed in sterile water, and planted in

[1] The writer is indebted to Mr. J. M. R. Adams for faithful assistance in the inoculation tests.

sterilized soil in the greenhouse, or in soil new to these crops in field plots. Soil inoculations were made by pouring a few cubic centimeters of a sterile-water spore suspension of the organism to be used on the steam-sterilized soil, either before or after planting the seed, or in the vicinity of the seedlings. The majority of the inoculations were made through wounds at the hypocotyl below the soil level. The wounds were made with a sterile scalpel, and after the inoculum was introduced, the soil was replaced to prevent drying. A few inoculations were made through wounds made by breaking off leaves and pods of the okra plants.

Approximately as many control plants were prepared as plants for inoculation. Half of these were wounded or otherwise subjected to the treatment employed on the plants inoculated, and the other half were left as unwounded controls. For the wounded controls, sterile water was used for inoculating, in lieu of the spore suspension. Wilting plants were examined microscopically for mycelium in the xylem of roots, stem, or petioles; and pure cultures of the organism used were reisolated and identified as a control on the work. Frequently such reisolated strains were used for subsequent inoculations.

In but one experiment was there any wilt in the controls. In this case after six weeks 6 per cent wilted, and the organism used in the experiment was recovered from the interior of the plants. However, this was a field test and there was in the duration of the experiment an opportunity for infection from inoculated plants in the adjoining rows on either side.

INOCULATION OF VARIOUS ECONOMIC PLANTS WITH VERTICILLIUM ALBO-ATRUM AND FUSARIUM VASINFECTUM

Inasmuch as the inoculation experiments were carried on in a uniform manner and controlled by a large number of wounded and unwounded plants, as well as by reisolation and identification of the organisms causing the disease, the results are comparable. For convenience these results are brought together in tabular form (Tables III, IV, V) and the discussion of the results of the inoculations are arranged according to the host plant and the parasite used.

HISTORY OF THE STRAINS OF VERTICILLIUM ALBO-ATRUM AND FUSARIUM VASINFECTUM USED FOR INOCULATION

The history of the various strains of *V. albo-atrum* and *F. vasinfectum* used for inoculating the several host plants is as follows:

F. vasinfectum strain 1855. Reisolated from the vascular system of a cotton plant, which was wilting as a result of inoculation with strain 1733, a reisolation of strain 1635, which was in turn a reisolation of the original strain 1485, isolated from the discolored vascular system of a wilting cotton plant at Florence, S. C., in 1912.

F. vasinfectum strains 2708, 2709, 3203. Isolated from the discolored vascular system of wilting okra plants collected at Florence, S. C. at

different times. Strain 3592 is a reisolation from an okra plant inoculated with strain 3203.

F. vasinfectum strain 3210. Isolated from the vascular system of an okra plant collected at Sumter, S. C.

V. albo-atrum strain 1717. Isolated from the interior of the stem of a wilting okra plant at Monetta, S. C. Strains 2943, 3075, 3076, and 3156 are reisolations from okra plants inoculated with strain 1717.

V. albo-atrum strain 2821. Isolated from wilting okra plant collected at Middle River, Cal.

V. albo-atrum strain 1685. Isolated from wilting plant of *Solanum melongena*.

V. albo-atrum strain 2784. Isolated from wilting potato plant.

V. albo-atrum strain 2985. Isolated from wilting snapdragon plant by Brown (*4*).

TABLE III.—*Results of inoculating okra plants with Verticillium albo-atrum from various hosts*

Number of plants	Age.	Locality.	Method of inoculation.	Species and strain of organism.	Incubation period.	Percentage ultimately wilting.	Time.
	Days.				*Days.*		*Days.*
40	17	Greenhouse.	Soil	*V. albo-atrum*, okra (1717).	12	88	21
60	(a)	do	do	do	40	59	60
60	(a)	do	do	*V. albo-atrum*, Irish potato (2784).	40	55	60
40	37	do	Wounds: hypocotyl.	*V. albo-atrum*, okra (1717).	11	100	30
16	46	do	do	do	13	81	30
12	90	do	do	do	18	100	30
13	30	do	do	do	12	92	30
17	46	do	do	*V. albo-atrum*, okra (3075).	15	66	30
17	46	do	do	*V. albo-atrum*, okra (3076).	13	17	30
40	46	do	do	*V. albo-atrum*, okra (2943).	13	40	30
9	90	do	do	*V. albo-atrum*, okra (2821).	18	100	24
40	46	do	do	*V. albo-atrum*, snapdragon (2985).	.....	0	30
40	46	do	Wounds: pedicel.	*V. albo-atrum*, okra (1717).	.....	0	30
26	30	do	do	do	.....	0	45
58	60	Field	Wounds: hypocotyl.	do	10	75	43
14	60	do	do	*V. albo-atrum*, okra (3156).	14	71	23
19	60	do	do	*V. albo-atrum*, eggplant (1685).	10	36	43
26	60	do	do	*V. albo-atrum*, Irish potato (2784).	10	73	43
22	60	do	do	*V. albo-atrum*, snapdragon (2985).	10	45	43

[a] Soil inoculated before planting the seed.

TABLE IV.—*Results of inoculating okra plants with Fusarium vasinfectum from cotton and okra*

Number of plants	Age.	Locality.	Method of inoculation.	Species and strain of organism.	Incubation period.	Percentage ultimately wilting.	Time.
	Days.				*Days.*		*Days.*
40	17	Greenhouse.	Soil..........	*F. vasinfectum*, okra (2709).	.....	0	56
40	17	.....do......	.....do........	*F. vasinfectum*, cotton (1855).	.....	0	56
40	37	.....do......	Wounds: hypocotyl.	.....do................	.....	0	56
99	21	.....do......	.....do........	.....do................	17	1	36
40	37	.....do......	.....do........	*F. vasinfectum;* okra (2709).	.....	0	56
40	37	.....do......	.....do........	*F. vasinfectum*, okra (2708).	.....	0	56
30	24	.....do......	.....do........	*F. vasinfectum*, okra (3203).	5	50	15
50	49	.....do......	.....do........	.....do................	.....	0	30
100	10	.....do......	.....do........	.....do................	6	55	18
20	60	.....do......	.....do........	*F. vasinfectum*, okra (3210).	.....	0	23
17	21	.....do......	.....do........	*F. vasinfectum*, okra (3592).	17	6	36

TABLE V.—*Results of inoculating cotton, eggplant, and Brabham cowpeas with the okra-wilt organisms through wounds at the hypocotyl*

Host.	Number of plants.	Age.	Locality.	Species and strain of organism.	Incubation period.	Percentage ultimately wilting.	Time.
		Days.			*Days.*		*Days.*
Cotton........	14	60	Field........	*F. vasinfectum*, okra (3210).	.....	0	23
Do.........	20	60	.....do.......	*V. albo-atrum*, okra (3156).	14	80	23
Do.........	18	21	Greenhouse..	*F. vasinfectum*, cotton (1855).	17	89	36
Do.........	43	21	.....do.......	*F. vasinfectum*, okra (3592).	17	87	36
Eggplant.......	7	45	.....do.......	*V. albo-atrum*, okra (1717).	10	85	15
Do.........	5	45	.....do.......	*V. albo-atrum*, snapdragon (2985).	10	80	15
Do.........	7	45	.....do.......	*V. albo-atrum*, eggplant (1685).	10	100	15
Brabham cowpeas.	16	24	.....do.......	*F. vasinfectum*, okra (3203).	.....	0	60

INOCULATION OF OKRA

No difficulty was experienced in securing typical wilting okra plants (Pl. 21, 22) by the inoculation of the soil or of the plants through wounds at the hypocotyl with pure cultures of *V. albo-atrum* isolated from okra, eggplant, potato, or snapdragon. By the inoculation of the soil in the vicinity of 17-day-old plants with strain 1717 isolated from okra, 88 per cent of wilting plants were obtained in 21 days. Inoculations of the soil with this strain before planting the seed resulted in 59 per cent of wilt. Inoculation of 37- to 90-day-old okra plants with this strain through the hypocotyl gave from 75 to 100 per cent of wilting plants. The reisolated strains of *V. albo-atrum* 1717 gave the following results: Strain 2943, 40 per cent; strain 3075, 66 per cent; strain 3076, 16 per cent; strain 3156, 71 per cent. *V. albo-atrum*, strain 2821, isolated from a wilting okra plant from Middle River, Cal., produced 100 per cent of wilting plants through hypocotyl inoculations.

The introduction of *V. albo-atrum* into wounds of the pedicel and the stem of okra plants was without apparent effect, thus indicating that there is no danger of carrying the wilt disease from plant to plant by the cutting knife.

The inoculation of okra plants with *V albo-atrum* from hosts other than okra gave the following results: Strain 2985, isolated from snapdragon, produced from none to 45 per cent of wilt through hypocotyl inoculation; strain 2784 from potato gave 73 per cent through hypocotyl wounds and 55 per cent through soil inoculation previous to planting the seed; strain 1685, isolated from eggplant, gave 43 per cent of wilt through the inoculation of 60-day-old plants at the hypocotyl.

The results of inoculating okra plants with *F. vasinfectum* were for some time disappointing. However, after an insight was gained into the conditions necessary to induce the Fusarium-wilt of okra, better results were obtained. With the species of Fusarium from okra in two trials, 50 and 55 per cent of wilting plants were secured by inoculation through the hypocotyl (Pl. 23). But with the species of Fusarium from cotton only 1 per cent was obtained. However, the cotton inoculated with the species of Fusarium (3592) from okra and the strain (1855) from cotton gave a high percentage of wilting plants in a parallel test of the two strains (Pl. 24–26).

INOCULATION OF COTTON

Fusarium vasinfectum strain 1855 from cotton, when introduced into wounds at the hypocotyl of 21-day-old plants, gave 50 per cent of wilt in 17 days and a total of 89 per cent in 5 weeks (Pl. 25). Similarly, in a parallel test, *F. vasinfectum* strain 3592 from okra gave 42 per cent of wilt in 17 days and a total of 87 per cent in 5 weeks (Pl. 26). In contrast to these results are those secured with these strains on okra in the parallel

test where but 1 and 6 per cent of wilt was secured. *Verticillium albo-atrum* strain 3156 inoculated through wounds of the hypocotyl gave 80 per cent of wilt in 14 days.

The Verticillium-wilt of cotton, as observed in these tests can not be distinguished from the very destructive Fusarium-wilt of this crop unless a critical examination be made. It is possible that this wilt is present in the cotton fields of the South and has been overlooked, owing to the similarity of the two diseases.

INOCULATION OF EGGPLANT

Eggplant was found to be susceptable to the strains of *V. albo-atrum* isolated from okra, eggplant (Pl. 20), and snapdragon. Strain 1717 from okra gave 85 per cent of wilt in 15 days; strain 1685 from eggplant gave 100 per cent in the same time, and strain 2985 from snapdragon 80 per cent correspondingly. The inoculations were made through wounds of the hypocotyl on 45-day-old plants.

INOCULATION OF BRABHAM COWPEAS

Twenty-four-day-old seedlings of Brabham cowpeas were inoculated through the hypocotyl with *F. vasinfectum* strain 3203 from okra. Although held under observation for 60 days, no cases of wilt were detected.

CONCLUSIONS

There are two similar wilt diseases of okra, caused, respectively, by *Fusarium vasinfectum* and *Verticillium albo-atrum.* These diseases can be differentiated only by isolating the causal fungi. The Fusarium-wilt in general is more serious in the southern range of okra growing, while the Verticillium-wilt is more serious in the northern range of this crop. However, the former has been reported in Connecticut, and the latter occurs in South Carolina and Alabama.

The wilt diseases of the Irish potato offer a parallel case of two organisms producing the same disease symptoms. Here, the Fusarium-wilt is induced by *F. oxysporum* and the Verticillium-wilt by *V. albo-atrum*, as in okra. Similarly, it is demonstrated in this paper for the first time that cotton may have both wilt diseases. No doubt there are several other economic plants, which act as host to vascular parasites of the genera Fusarium and Verticillium—for example, China aster, ginseng, eggplant, brambles.

V. albo-atrum was isolated from the discolored vascular system of wilting plants of okra, eggplant, potato, and species of Abutilon and Xanthium, and was demonstrated in these studies to be the cause of a wilt disease at least of okra, eggplant, and cotton. It was identified from a wilt disease of snapdragon after its causal relation to this disease had been established by Miss Nellie A. Brown, of the Bureau of Plant

Industry. Okra is susceptible to inoculation with *V. albo-artum* from okra, snapdragon, eggplant, and potato; and to *F. vasinfectum* from okra. Eggplant is susceptible to *V. albo-atrum* from eggplant, okra, and snapdragon. Cotton is susceptible to the strains of this fungus from okra and to *F. vasinfectum* from okra and cotton.

Thus, it is apparent that the species of Fusarium causing the wilt disease of okra is identical with *F. vasinfectum*. Likewise it is evident that *V. albo-atrum* is a serious vascular parasite of a number of different economic plants. In view of the fact that the genus Acrostalagmus should be combined with the earlier genus Verticillium, it seems probable that *V. albo-atrum* is the fungus described by Van Hook under the name "*Acrostalagmus albus*," the cause of ginseng-wilt; by Lawrence as *A. caulophagus*, the cause of the bluestem disease of black raspberry; and by Gueguen as *A. vilmorinii*, the cause of a wilt disease of China aster and associated with a disease of cacao fruits.

V. albo-atrum and *F. vasinfectum* are readily cultivated artificially, indicating that both are capable of persisting saprophytically in the soil for an indefinite period in the absence of the preferred host. There was no indication that either fungus is carried from field to field or from year to year by the seed or from plant to plant by the cutting knives.

As a control measure it may be suggested that seed be selected only from healthy plants. If extreme precaution is to be taken, the seed may be disinfected in a formalin solution (1 to 240) for two hours.

Since okra, eggplant, potato, cotton, snapdragon, and the weeds *Xanthium* spp. and *Abutilon* spp. are all susceptible to the Verticillium-wilt, as well as ginseng, China aster, and black raspberry, as seems probable, these plants should be taken into consideration in planning a rotation to eliminate wilt diseases. Similarly, okra and cotton are hosts of *F. vasinfectum* and should not follow each other in rotation if best results are expected.

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PLATE A

Fusarium vasinfectum on vegetable media:

1–3.—Growth on steamed potato. Both potato cultures show pionnotes.
2, 4.—Growth on rice.
Cultures 1 and 2 were grown in a strong north light; 3 and 4 in a subdued light.

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PLATE 17

A–H.—*Verticillium albo-atrum:*

A, Simple conidiophores and conidia. ×1,000.

B, Same showing, respectively, the collection of the conidia on the sterigma in irregular aggregations in dry air, and in water drops in humid air. ×1,000.

C, Verticillate conidiophores bearing one and three whorls, or virtels, of branches, respectively. ×500.

D, Verticillate conidiophore having conidial heads, from humid environment—that is, moisture drops in which the conidia float as in figure B. This is the so-called Acrostalagmus type of conidial head. ×500.

E, Mycelium of *V. albo-atrum* in the vascular ducts of an okra plant inoculated with this fungus. ×250.

F, H, Germinating conidia: F, ×500; H, ×1,000.

G, Swollen, sclerotia-like mycelium. ×500.

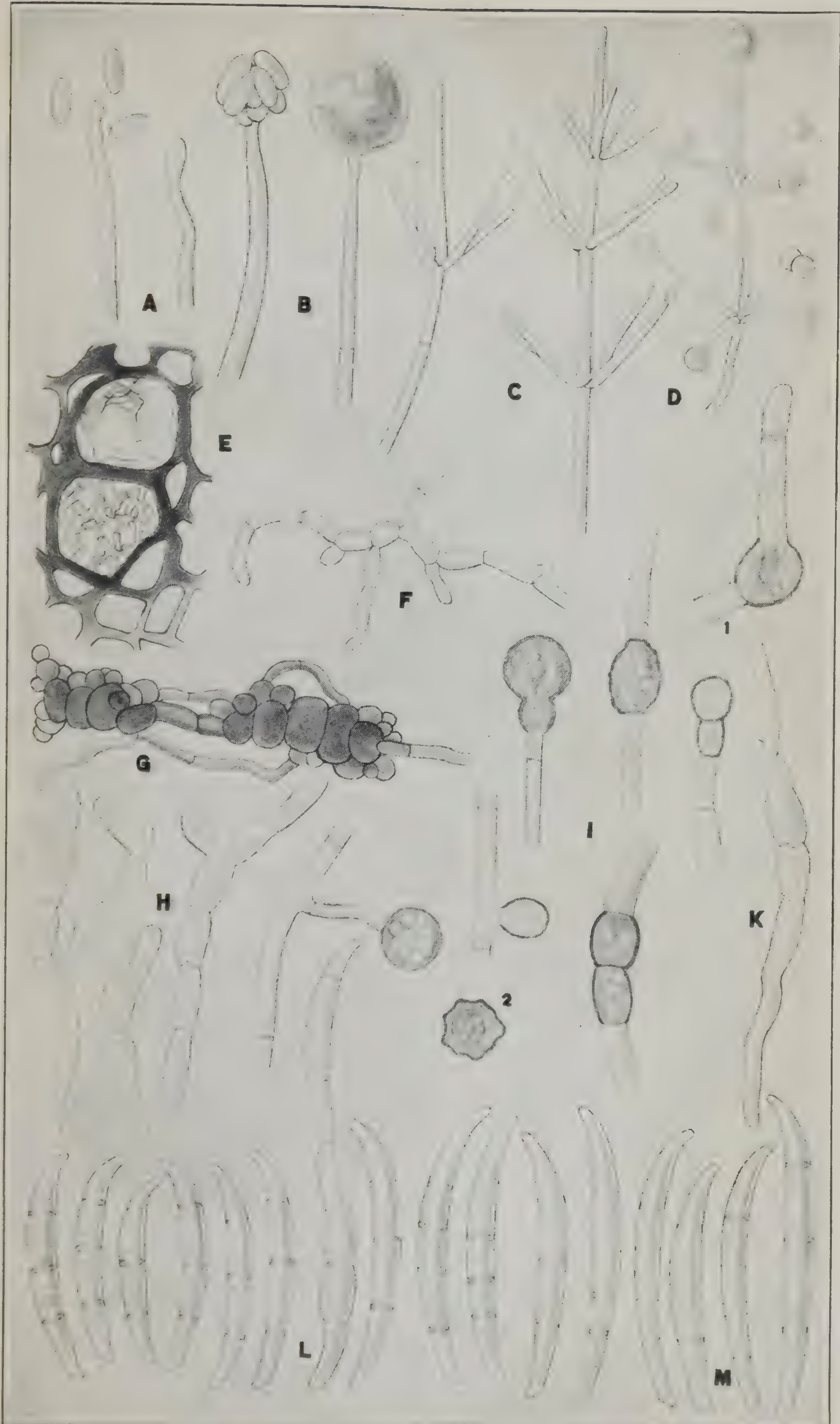
I–M.—*Fusarium vasinfectum:*

I, Terminal, intercalary and conidial chlamydospores. 1, Germinating terminal chlamydospore. 2, Free mature chlamydospore. ×1,000.

K, Germinating macroconidium. ×1,000.

L, *F. vasinfectum* from okra-wilt. Twelve macroconidia. ×1,000.

M, *F. vasinfectum* from cotton-wilt. Four macroconidia. ×1,000.


A
B
C
D
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F
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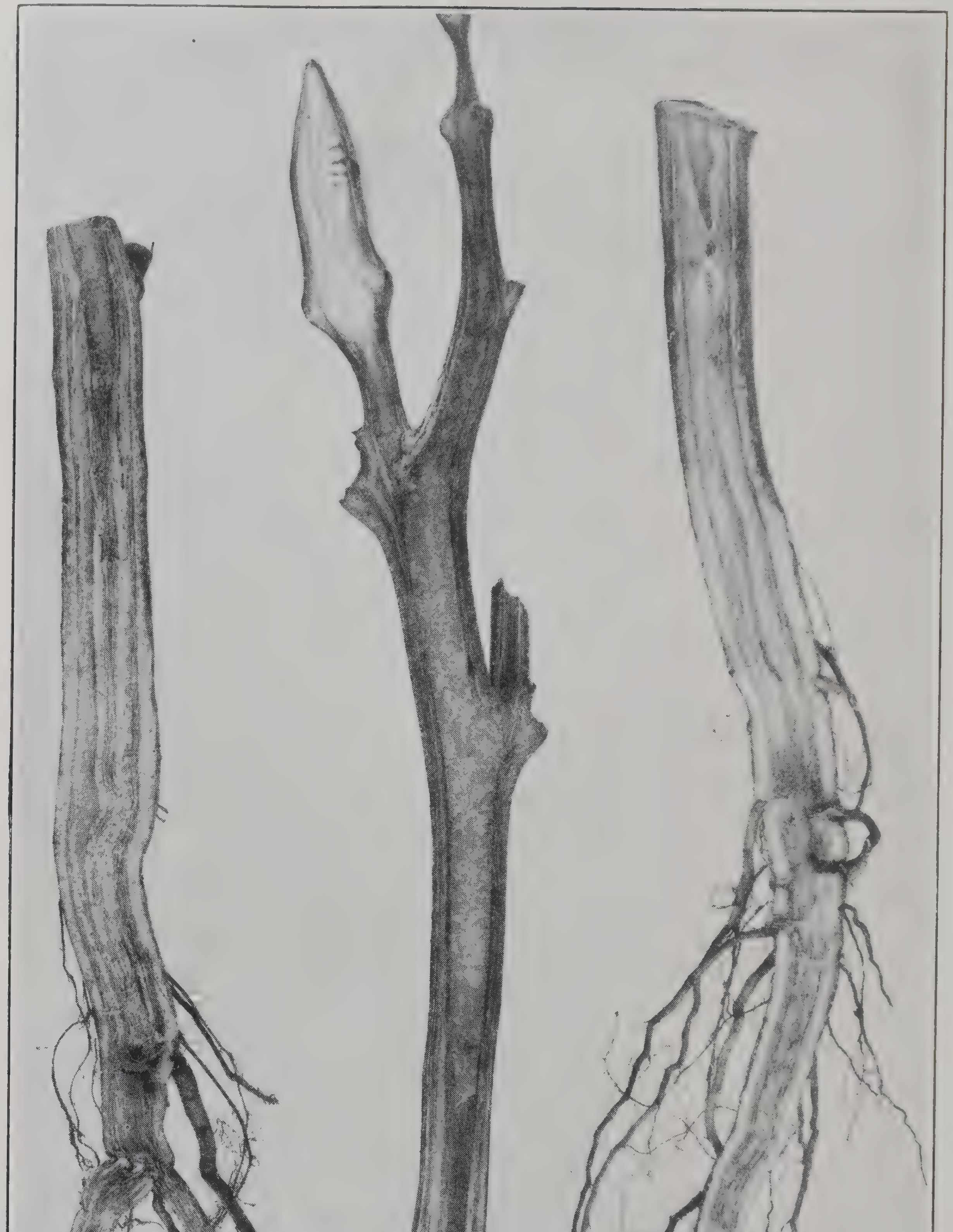


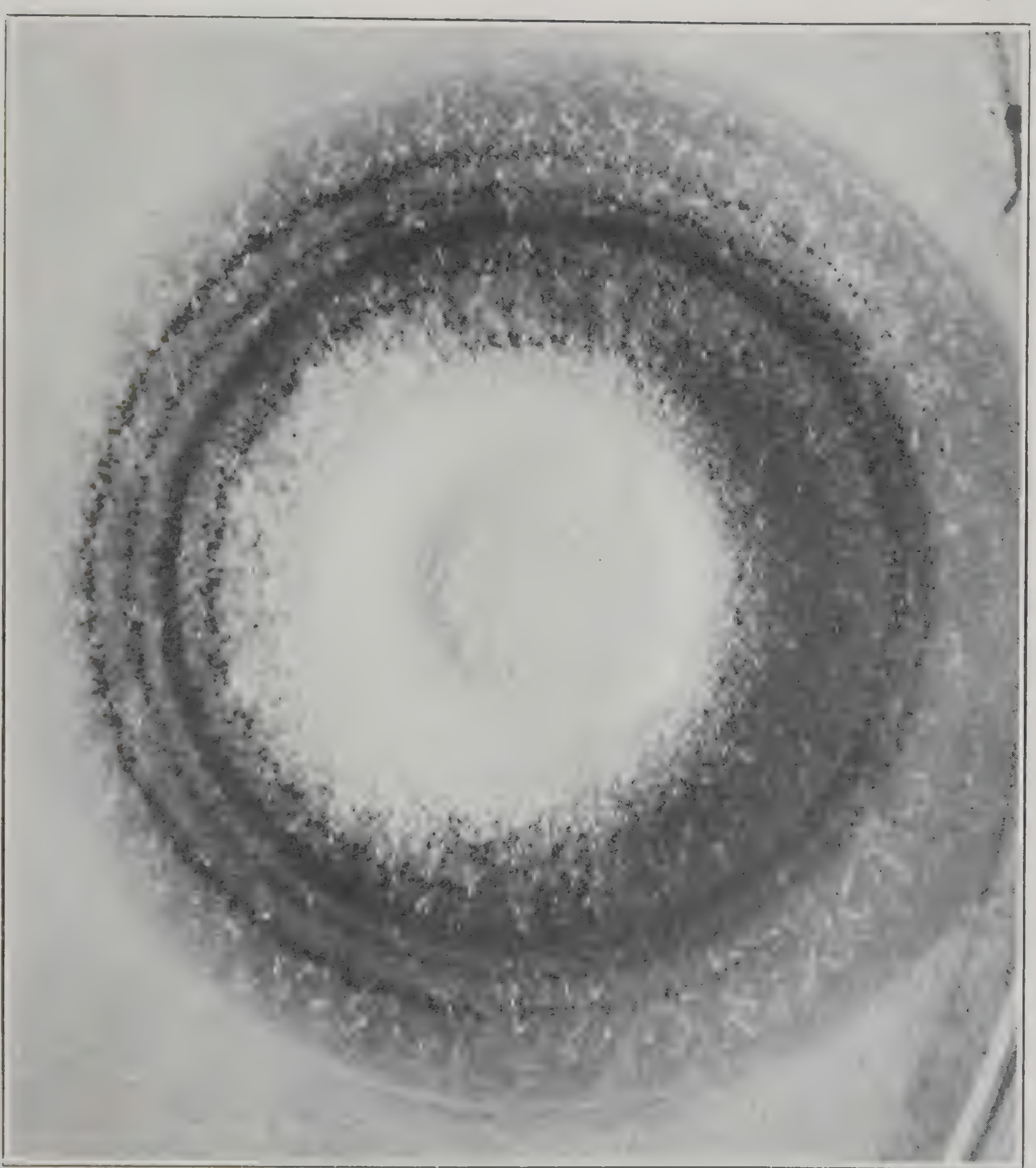
PLATE 18

Longitudinal section of an okra plant naturally infected with *Verticillium albo-atrum*, showing the typical appearance. The vascular elements are discolored from the roots to the pedicels and petioles. About natural size.

PLATE 19

Verticillium albo-atrum:

Two-weeks-old colony on potato agar, showing the concentric rings of black sclerotial bodies. ×4.




B
A

PLATE 20

Solanum melongena, showing effect of wilt:

A.—Control plant of the same age as the wilted plant (B). Photographed at the same time as figure B. ×2/5.

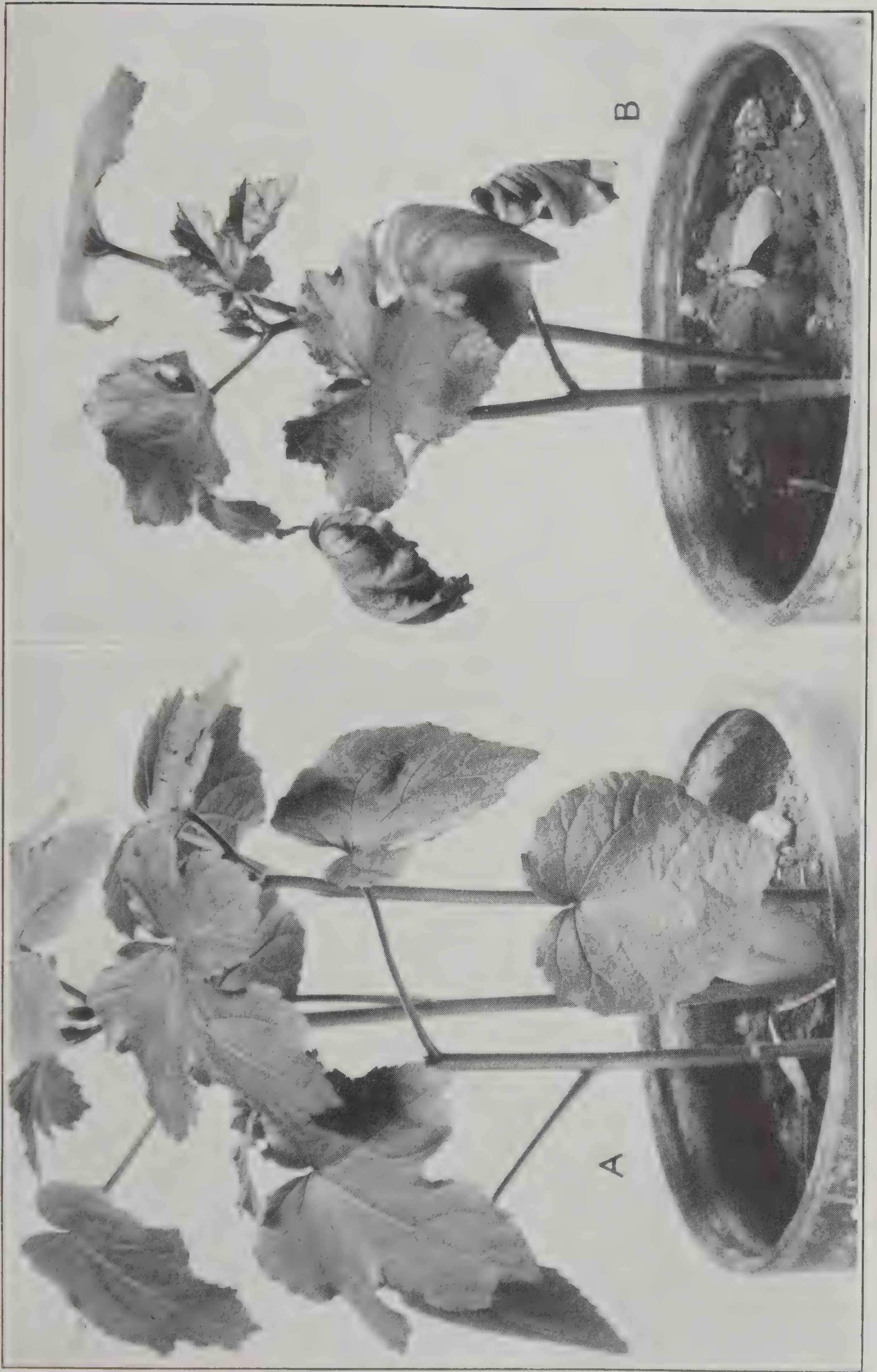
B.—Wilted plant photographed two months after inoculation at the hypocotyl with *Verticillium albo-atrum* isolated from wilted eggplant. The organism was recovered from the stem of the small plant 10 cm. above the point of inoculation. ×2/5.

PLATE 21

Abelmoschus esculentus, showing effect of wilt:

A.—Control plant. Photographed at the same time as figure B. × (about) 1/2.

B.—Wilted plant photographed two weeks after inoculation at the hypocotyl with a pure culture of *Verticillium albo-atrum*. × (about) 1/2.


A
B


A
B

PLATE 22

Abelmoschus esculentus, showing effect of wilt:

A.—Wilted plant inoculated with *Verticilium albo-atrum*. × (about) 1/3.

B.—Control plant of the same age as wilted plant. × (about) 1/3.

Both plants were photographed two months after the wilted plant had been inoculated.

PLATE 23

Abelmoschus esculentus, showing the effect of wilt as a result of inoculation with *Fusarium vasinfectum* isolated from okra-wilt. The inoculation was made at the hypocotyl and the organism was recovered from the vascular tissues of the petioles two weeks afterwards—that is, immediately after the photograph was taken. ×2/3.

PLATE 24

Gossypium herbaceum (Columbia variety):

Control plants 35 days old. Photographed 15 days after having been wounded at the hypocotyl. Natural size.

PLATE 25

Gossypium herbaceum (Columbia variety), showing effect of wilt:

Wilting plants photographed 15 days after inoculation at the hypocotyl with *Fusarium vasinfectum*, strain 1855, isolated from wilting cotton plants. The plants are the same age as the control plants in Plate 24. Natural size.


16

PLATE 26

Gossypium herbaceum (Columbia variety), showing effect of wilt:

Wilting plants photographed 15 days after inoculation at the hypocotyl with *Fusarium vasinfectum*, strain 3592, isolated from wilting okra. The plants are the same age as those in Plates 24 and 25. Natural size.

PLATE 27

Abelmoschus esculentus, showing the characteristic symptoms of the wilt produced by *Verticillium albo-atrum*. Photographed in a field in New Jersey.

WINTER CYCLE OF EGG PRODUCTION IN THE RHODE ISLAND RED BREED OF THE DOMESTIC FOWL

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INTRODUCTION

The winter cycle of egg production is one of the internal factors concerned in determining total production. It was first recognized by Pearl and Surface in Barred Plymouth Rocks. They state (*7, p. 99–100*):[1]

> It will undeniably be advantageous, in studying certain phases of the problem of egg production, to endeavor to use a time unit which conforms to the *natural periodicity* displayed by hens [italics are mine—H. D. G.]. . . .
>
> The plan followed at the present time in the investigations in progress at the Maine Station breaks the laying year up into four parts. The first of these includes the months of November, December, January, and February. Broadly speaking, this is the period of winter laying and is so designated. . . .
>
> The justification for the conclusion that this division of the year is in general a natural one and corresponds to a real cyclical periodicity in egg production is in considerable measure to be found in the facts regarding mean monthly egg production and variation in this character set forth in this and the preceding section of the paper. The winter-laying period is a period characterized by rapid increase in mean production associated with a relatively equally rapid decrease in variability, both absolute and relative. In this period a large part of the flock falls in the A component of the monthly distributions (see p. 142). This laying period is, strictly speaking, not a part of the natural or normal reproductive cycle of the hen. Egg laying in this part of the year is something which during domestication has been added on, as it were, to the natural reproductive activity of the wild *Gallus*. It is a result of "forcing" or special stimulation. From the evolution standpoint, egg production in these months is a comparatively recent acquisition. Such being the case, the greater variability observed in winter laying is only what would be expected.
>
> The limits of this artificial winter cycle of egg production are fairly well defined. It begins with the beginning of the laying year. Its other limit is marked by the slacking up in egg production, which occurs in February (see Fig. 1). This slackening up in February, which appears to be a characteristic of egg production, generally, is to be explained, we believe, chiefly if not entirely as the result of the ending of the winter cycle by the majority of birds which have laid during the early winter. Such birds rest for a period at about this time before beginning the spring laying cycle. Of course it must be understood that these statements are made only with reference to what might be called the general or average course of events. Particular birds may form exceptions in their laying. Many birds, of course, have no proper winter cycle of laying at all. They begin to lay for the first time in January or February, and keep on laying without any large break straight through the spring cycle.

[1] Reference is made by number (italic) to "Literature cited," p. 574.

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In discussing the monthly egg distribution Pearl and Surface (7, *p. 89–90*) state:

Considering the form of the polygon somewhat more in detail, we note that the line starts from a low point in November and rises rather rapidly and in almost a straight line to January. The slope of the line from January to February is downward. In other words, there is an indentation in the ascending limb of the egg-production polygon in the month of February. This is a very characteristic feature of the distribution of egg production, not only observed with the birds here under discussion but also in published records from other sources.

A study of Table 1 shows that this is generally true for every year covered by the investigation. While the February mean production is not necessarily lower than the January, though this is true in many cases, there is a perceptible slowing of the rate of increase in egg production which has obtained up to that time. The most probable interpretation of this appears to us to be that the February indentation in the egg-production curve represents a rest or reaction after the winter laying and in anticipation of the heavy March and April production. It marks the completion of a laying cycle on the part of those birds which have been laying during the winter months.

The mean egg production for February, however, seems to rest on a basis of 28 days. If reduced to a basis of 31 days, the mean production is 12.03 eggs, a value, however, that produces a pronounced indentation in the upward slope of the polygon. Pearl believes that this change in the slope of the polygon, disregarding entirely any actual drop in production, is indicative of a winter cycle. However, a change in the slope of such a polygon would occur if a flock of birds began to lay in some given month, gradually increased in production for a definite period of some length until they reached a maixmum and maintained this maximum for a period of several months. Pearl, moreover, is inclined to believe that the change in mean temperature that occurs in March bears no causal relation to the increase in egg production that is observed at this season. Now, while it is clear that there are other factors than the change in temperature that increases production at this season, it is not at all clear that temperature can be entirely eliminated as a factor. In the imaginary case just mentioned it seems possible that the maximum production under one set of environmental conditions might be different from that observed under another set. Hence, it seems entirely probable that the maximum production possible in January and February would not be as high as in March, and that the indentation noted in the curve of production merely means that the maximum production possible for midwinter conditions has been reached.

In several other papers, notably those of Pearl (*5, 6*), the winter cycle is again discussed. In the former paper he states (*5, p. 173–174*):

(2) The upper limit of the winter period at March 1 is arbitrary, and only approximately coincides with the biological reality. Actually with most birds the spring or reproductive cycle of production (cf. 37) begins in the latter part of February. In handling the material it has been found necessary (for reasons which will be

obvious upon consideration of the matter) to take a fixed date for the beginning of the spring cycle of laying and the ending of the winter cycle. The records of the station prior to 1908 are tabulated only for months (the daily records unfortunately having been destroyed before I took charge of the work), and on this account it is necessary to take the working limit of the winter cycle at the end of a calendar month. Since March 1 comes the nearest to the biological limit of any date which is also the beginning of a calendar month it has been chosen. The error introduced by taking this arbitrary date for a point which really shifts within rather narrow limits is, on the average, small. However, it must be recognized as a disturbing element in the individual case. Thus, some birds which really lack any genetic factor for winter production will begin to lay in the last days of February, and consequently on the arbitrary "March 1" basis will actually be credited with a small winter production. This will tend to make the number of zero birds observed smaller than that expected on theory.

In the latter paper, entitled "Measurement of the Winter Cycle in the Egg Production of Domestic Fowl," a comparison is made between the egg production of a pullet during the first 300 days of its life with its egg production up to March 1 of the pullet year. He finds that there is very little difference in the value of these two measures of production

The evidence for a winter cycle published by Pearl is all mass evidence. The possibility that a flock might be heterogeneous in respect to the winter cycle is not considered by him. It is true that he speaks of particular birds forming exceptions to the rule in their laying, but the sentence immediately following seems to indicate that Pearl has in mind birds that begin to lay late in the winter (*7, p. 100*) rather than birds that lay throughout the winter. However, a few records that form real exceptions to the rule can be found in the records of individual Barred Plymouth Rocks published by Gowell (*3, 4*).

Pearl's mass evidence of the existence of a winter cycle is supported by a study of the records described below. While a cursory examination of the data on egg production of Rhode Island Reds at this Station indicates that a considerable percentage of our records are without visible indications of a winter cycle (since many birds that begin to lay in November continue to lay without noticeable slackening of production straight through the winter and spring), at the same time there are many instances in which the existence of a winter cycle is indisputable. Records of both sorts may also be observed in the reports from various egg-laying contests. Further, an examination of Gowell's records of the monthly egg production of individuals (*3, 4*) shows in most instances a marked decrease in the production for February over January, indicating that the birds either stop laying entirely for a time during one or both months or that they slow down in their daily rate. The former alternative, in view of Pearl's statements, appears to represent the facts. A few records published by Miss Curtiss in another connection also show the same thing.

Since inspection of our records shows the possibility of the existence of two types of birds with respect to a winter cycle—namely (1) those exhibiting such a cycle, and (2) those that lay but give no evidence of a cycle—it becomes necessary to examine the matter in detail, and especially to endeavor to discover some criterion by which any individual may be suitably classified. This has been attempted by a study of individual records associated with a study of the length and seasonal distribution of pauses in production as well as a study of the monthly rate of production.

MATERIAL AND METHODS

The material studied consisted of the daily egg records of three flocks of Rhode Island Red pullets, hatched, respectively, in 1913, 1915, and 1916. While Pearl has never published individual records bearing on this point, Gowell, who initiated the work at the Maine Station, has published the individual monthly egg records of the flocks making their pullet records in 1899–1900, 1900–1901, 1901–2 (*3, 4*). These have been used for comparison with our records. Further comparisons are outside the scope of this paper.

It is obvious that a cycle in production should appear on the record in one of two ways. Either there is a period of continuous egg production followed by a period in which no eggs whatsoever are laid, or else the period of production is followed by a period in which eggs are produced at a less rapid rate than previously. It is well known to poultrymen that hens often lay well for a while and then enter on a resting period of variable length. The egg-producing period may be designated as a "litter." This is, of course, one form of cycle, but is to be distinguished in most cases from the winter cycle through the fact that the period of egg production is in the latter relatively long and may be composed of more than one litter. As a rule the only difficulty encountered is the case where a single litter extends over the entire winter. In this instance one can not tell whether one has a winter cycle, or a very long litter, or whether the two coincide.

Since any cycle consists of a productive phase and a nonproductive phase, we have put the original records into tabular form (Table I), to which the reader's attention is directed in lieu of a detailed account of individual histories and variations. In studying this table we have come to pay particular attention to the pauses in production, since these serve as our visible indexes of cycles.

EXPLANATION OF TABLE I

In Table I the details of the records of several families are shown. Practically all the different sorts of pauses are illustrated. Pauses of less than three days in length have not been included. The arrangement of the tables by families brings out the strong resemblances between the members of the various families. The record of the mother is printed in italics at the head of the list of her daughters.

Daughters that became broody before March 1 are given in bold-faced type. Among other things listed in the column headed "Remarks," pauses occurring after March 1 and extending through April 30 are listed and are to be read as follows: The date of the beginning of a pause is given, followed by its length, and in case more pauses occur it is indicated by the number of eggs (inclosed in parentheses) followed by the length of the succeeding pause. Thus, No. 9277 in the family of male 3617 by female 6003, a pause of 7 days length began April 7, when the bird laid one egg followed by a 3-day pause.

Families sired by male 3617.—The progeny of one father and two mothers. None of the members of these families shows a well-defined winter cycle. The February egg production, in most cases where production began January 1 or before, is greater than for January. The families are also noteworthy because of the number of birds without any pause exceeding two days in length.

Family sired by male 5240.—In this family several individuals have a well-defined winter cycle; others lack such a cycle.

Family sired by male 4786.—This family is characterized by the existence of a single short pause in each record.

Family sired by male 6781.—This family is characterized by numerous pauses of varying lengths.

Family sired by male 4723.—This is a heterogeneous family.

TABLE I.—*Details of the winter egg production of several families of Rhode Island Reds*

TWO FAMILIES SIRED BY MALE 3617

Band No.	Date hatched.	Date of first egg.	Number of eggs to Mar. 1.	Number of pauses.	Total length of time spent in pauses.	Length of each pause.	Date of first and last day of each pause.	Number of eggs between pauses.	Number of eggs before the first pause.	Average length of pauses.	Average number of eggs between pauses.	February production greater or less than January.	Egg production. December.	Egg production. January.	Egg production. February and first 3 days of March.	Egg production. Remainder of March.	Remarks.
					Days.	*Days.*				*Days.*							
6003	*1915.* *Apr. 25*	*Dec. 6*	*55*	*0*								=	*16*	*20*	*20*	*18*	
8748	*1916.* May 7	Nov. 30	56	1	3	3	Jan. 1–3		22			=	21	18	18	18	
8749	...do	Nov. 24	60	2	7	3 4	Feb. 9–11 Feb. 24–27	7	52	3.5		− 6	22	20	14	18	
9024	May 14	Dec. 31	41	0								+ 3	1	20	23	21	Apr. 27–3.
9276	May 21	Dec. 22	39	0								+ 3	6	16	19	16	Mar. 22–4.
9021	May 14	Nov. 27	71	0								+ 3	23	21	24	20	
9023	...do	Dec. 7	59	0								− 1	18	22	21	27	
9277	May 21	Jan. 20	27	0								+15	0	7	22	[illegible]	Apr. 7–7(1)3.
9279	...do	Feb. 28	1	0								+ 2	0	0	2	18	
9439	May 28	Jan. 19	23	0								+11	0	7	18	18	
9441	...do	Jan. 23	25	0								+18	0	5	23	6	Mar. 7–20.
4716	*1915.* *Mar. 14*	*Nov. 22*	*75*	*0*								*+ 1*	*21*	*24*	*25*	*13*	*Mar. 15–10.*
8403	*1916.* Apr. 23	Dec. 3	59	1	3	3	Dec. 4–6		1			− 1	17	23	22	16	Mar. 4–3.
8404	...do	Nov. 5	97	0								− 5	25	29	24	14	
8508	Apr. 30	Nov. 1	70	8	27	4 3 4 3 3 4 3 3	Nov. 16–19 Nov. 26–28 Dec. 7–10 Dec. 14–16 Dec. 18–20 Jan. 7–10 Jan. 17–19 Feb. 4–6	5 5 3 1 11 4 11	12	3.4	5.7	+ 9	15	15	24	22	Mar. 20–3.
8509	...do	Dec. 1	61	0								+ 1	22	20	21	21	
9039	May 14	Jan. 1	36	0								+ 1	0	19	20	20	Apr. 27, inc.
9041	...do	Nov. 25	77	0								+ 1	26	24	25	25	
9042	...do	Jan. 10	32	0								+ 6	0	14	20	12	
9444	May 28	Jan. 5	37	0								+ 3	0	18	21	23	
9446	...do	Dec. 13	58	1	3	3	Dec. 25–27		9			+ 1	12	24	25	17	
8740	**May 7**	**Dec. 2**	**55**	**1**	**28**	**28**	**Feb. 6–Mar. 5**		**55**			**−21**	**24**	**26**	**5**	**23**	

FAMILY SIRED BY MALE 5240

6404	*1915. Mar. 9*	*Dec. 7*	*53*	*1*	*18*	*18*	*Feb. 19–Mar. 7*	....	*53*	....	....	*− 9*	*20*	*21*	*12*	*22*	*Pause due to broodiness.*
	1916.																
7625	Mar. 12	Sept. 20	110	0	....	....	....	....	....	....	....	+ 3	20	18	21	18	
8148	Apr. 16	Oct. 5	106	1	23	23	Jan. 25–Feb. 16	....	96	....	....	− 8	27	21	13	10	Mar. 8–16.
						4	Oct. 7–10	52									
8424	Apr. 23	Oct. 6	100	3	10	3	Dec. 18–20	32	1	3.3	42	+ 1	21	21	22	17	Mar. 6–6.
						3	Feb. 6–8	....									
						3	Oct. 28–30	82									
8559	Apr. 30	Oct. 19	89	3	22	9	Feb. 13–21	1	6	7.3	41.5	−10	25	21	11	18	
						10	Feb. 23–Mar. 4	....									
8560	...do.....	Oct. 23	87	1	3	3	Oct. 26–28	....	2	....	....	+ 1	24	20	21	20	
8561	...do.....	Nov. 21	38	1	40	40	Jan. 1–Feb. 9	....	25	....	....	+15	20	0	15	22	
8733	May 7	Nov. 5	85	0	....	....	....	....	....	....	....	− 4	22	24	20	20	

FAMILY SIRED BY MALE 4786

5881	*1915. Apr. 25*	*Dec. 14*	*66*	*0*	....	....	....	....	....	....	....	*− 1*	*14*	*28*	*27*	*26*	
	1916.																
7870	Apr. 2	Nov. 3	93	0	....	....	....	....	....	....	....	− 2	26	24	22	20	
8121	Apr. 16	Nov. 1	95	1	4	4	Nov. 2–5	....	1	....	....	− 1	27	26	25	17	
8395	Apr. 23	Nov. 19	58	1	10	10	Dec. 12–21	....	14	....	....	=	12	20	20	23	On floor Dec. 29.
8396	...do.....	Nov. 17	69	1	5	5	Dec. 12–16	....	16	....	....	+ 3	18	21	24	22	
9028	May 14	Dec. 8	56	1	3	3	Jan. 2–4	....	17	....	....	+ 2	16	20	22	20	Dec. 8 on floor.
9215	May 21	Jan. 23	25	1	4	4	Jan. 24–27	....	1	....	....	+21	0	3	24	22	Apr. 5–4. On floor Jan. 7–23.
8397	**Apr. 23**	**Nov. 21**	**62**	**1**	**25**	**25**	**Jan. 11–Feb. 4**	....	**43**	....	....	**+14**	**27**	**8**	**22**	**24**	
9029	**May 14**	**Nov. 27**	**56**	**1**	**25**	**25**	**Jan. 5–29**	....	**30**	....	....	**+22**	**24**	**5**	**27**	**22**	

TABLE I.—*Details of the winter egg production of several families of Rhode Island Reds*—Continued

FAMILY SIRED BY MALE 6781

Band No.	Date hatched.	Date of first egg.	Number of eggs to Mar. 1.	Number of pauses.	Total length of time spent in pauses.	Length of each pause.	Date of first and last day of each pause.	Number of eggs between pauses.	Number of eggs before the first pause.	Average length of pauses.	Average number of eggs between pauses.	February production greater or less than January.	Egg production. December.	Egg production. January.	Egg production. February and first 3 days of March.	Egg production. Remainder of March.	Remarks.
					Days.	*Days.*				*Days.*							
5554	1915 Apr. 11	Dec. 13	35	4	31	5	Jan. 10–14	2	20	7.8	5	+ 5	14	8	13	18	
						13	Jan. 19–31	1									
						3	Feb. 2–4	12									
						10	Feb. 27–Mar. 7	....									
7550	1916. Mar. 5	Sept. 10	59	2	81	74	Nov. 23–Feb. 4	1	48	40.5	....	+ 13	0	0	13	17	
						7	Feb. 6–12	....									
7623	Mar. 12	...do....	36	4	124	20	Sept. 11–30	2	1	31	11.7	+ 2	5	0	2	22	On floor Oct. 11.
						16	Oct. 3–18	1									
						4	Oct. 20–23	32									
						84	Dec. 7–Feb. 28	....									
7730	Mar. 19	Dec. 3	28	5	41	11	Dec. 29–Jan. 8	7	15	8.4	2.8	=	15	7	7	17	
						10	Jan. 22–31	1									
						4	Feb. 2–5	1									
						4	Feb. 7–10	2									
						12	Feb. 14–25	....									
8355	Apr. 23	Nov. 3	31	4	78	12	Nov. 4–15	1	1	19.5	1	+ 20	2	5	25	23	On floor Nov. 3–16; Dec. 6–12; Jan. 24. Apr. 11–3 (9) 4 (1) inc.
						19	Nov. 17–Dec. 5	1									
						5	Dec. 7–11	1									
						42	Dec. 13–Jan. 23	....									
8356	...do.....	Oct. 30	41	6	51	11	Oct. 31–Nov. 10	2	1	8.5	4.6	+ 4	8	14	18	19	On floor Oct. 30; Nov. 11; Dec. 10–15.
						25	Nov. 15–Dec. 9	1									
						4	Dec. 11–14	5									
						4	Dec. 24–27	6									
						4	Jan. 7–10	9									
						3	Jan. 27–29	....									
8359	...do.....	Oct. 25	38	4	70	22	Nov. 21–Dec. 12	6	17	17.5	5	− 1	7	8	7	18	On floor Dec. 13–15. Mar. 2–5.
						3	Dec. 22–25	1									
						22	Dec. 26–Jan. 16	8									
						23	Jan. 27–Feb. 18	....									
8594	Apr. 30	Dec. 10	32	3	27	3	Dec. 14–16	6	3	9	4.5	+12	10	6	18	19	On floor Dec. 10.
						3	Dec. 28–30	3									
						21	Jan. 4–24	....									

FAMILY SIRED BY MALE 4723

4178	*1914. June 1*	*Jan. 20*	*28*	*0*	....	....	....	....	....	....	....	*+12*	*0*	*9*	*21*	*20*	*Apr. 4-5.*
8069	1916. Apr. 9	Oct. 9	55	5	46	31	Dec. 7-Jan. 6......	7	33	9.2	4.3	− 3	3	13	10	18	Apr. 9-3.
						3	Jan. 17-19.........	7									
						3	Feb. 3-5...........	1									
						6	Feb. 7-12..........	2									
						3	Feb. 16-18.........	....									
8222	Apr. 16	Oct. 19	25	7	93	7	Oct. 22-28.........	2	2	13.3	3.2	− 2	0	8	6	16	
						3	Nov. 1-3..........	1									
						3	Nov. 5-7..........	8									
						53	Nov. 20-Jan. 11...	3									
						3	Jan. 18-20.........	2									
						3	Jan. 24-26.........	3									
						21	Feb. 1-21..........	....									
8223	...do.....	Oct. 8	81	1	11	11	Dec. 28-Jan. 7.....	....	52	....	....	+ 9	18	11	20	20	
8320	Apr. 23	Mar. 15	0	....	....	....	....	....	....	....	....	....	0	0	0	14	
8627	Apr. 30	Nov. 26	19	1	59	59	Dec. 27-Feb. 23...	....	16	....	....	+ 5	13	0	5	20	
8630	...do.....	Dec. 4	56	3	10	4	Dec. 9-12..........	36	3	3.3	18.5	− 4	16	23	19	22	
						3	Feb. 1-3...........	1									
						3	Feb. 5-7...........	....									
8913	May 14	Nov. 24	65	0	....	....	....	....	....	....	....	− 1	21	21	20	19	
8914	...do.....	Nov. 6	31	4	69	7	Nov. 7-13..........	3	1	17.3	10	+ 5	19	0	5	16	
						5	Nov. 18-22.........	24									
						54	Dec. 31-Feb. 22...	3									
						3	Feb. 27-Mar. 1....	....									
8917	...do.....	Feb. 22	5	0	....	....	....	....	....	....	....	+ 7	0	0	7	23	
9194	May 21	Dec. 8	20	1	50	50	Dec. 29-Feb. 16...	....	13	....	....	+ 9	13	0	9	21	

As inspection of these records shows that there are pronounced family resemblances in the number and length of pauses among the members of each of several families, and since environmental influences have been excluded, as far as possible, it is evident that our material is not homogeneous from the biometrical standpoint. Consequently most of the usual biometrical constants have not been calculated, since in this case such constants tend to conceal the differences we are endeavoring to find.

MEANS BY WHICH A WINTER CYCLE MAY BE RECOGNIZED

A.—PAUSES

LENGTH AND SEASONAL DISTRIBUTION

Two questions arise in regard to the length of the pauses: First, is there any significant difference in the length of pauses? Second, is there any difference in the distribution of pauses of varying length through the winter? Table II shows the percentage distribution of pauses beginning at two days in length, grouped according to the month of origin. The combined values for the three years are shown in the last column. It is clear that the pauses fall into two classes, short and long, the dividing line between the two groups falling at about 10 days. Further, it is evident from the table that the long pauses originate mainly in December and January, and to a less extent in February, but that the short pauses are distributed in a fairly uniform manner throughout the winter.[1] An examination of the pauses occurring in March and April not due to broodiness shows that while all pauses are less numerous than in the winter, and while a few long pauses may be found, most are short ones occurring in practically the same proportions observed in the other months.

[1] The increase in long pauses originating in December and January automatically decreases the percentage of the short pauses so that relatively they are somewhat less numerous in spite of the percentages being about equal.

TABLE II.—*Length of pauses distributed according to month in which they originate, for 1913–14, 1915–16, 1916–17* [a]

Length of pause.	1913–14					1915–16				
	November.	December.	January.	February.	March.	November.	December.	January.	February.	March.
Days.	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
2	75.00	68.63	51.54	53.33	48.81	71.15	57.02	53.85	53.81	59.69
3		7.84	19.23	20.00	13.09	9.62	14.91	8.24	15.71	12.25
4		17.65	6.15	9.17	13.09	3.85	3.51	4.95	8.57	7.14
5		1.96	3.85	4.17	4.76		2.63	3.85	1.90	8.16
2–5	75.00	96.08	80.77	86.66	79.76	84.62	78.07	70.88	80.00	87.25
6–10		3.92	4.62	5.83	10.71	1.92	5.26	6.59	5.71	6.12
11–15	12.50		3.08	2.50	1.19			3.30	2.86	3.06
16–20			1.54	1.67	3.57	1.92	.88	3.30	2.38	.51
21–25			3.85	2.50	1.19	1.92	1.75	2.20	1.90	1.02
26–30				.83	2.38	3.85	.88	2.20	3.81	1.02
31–35			6.15				2.63	1.10	1.43	
36–40	12.50					1.92	.88	1.65	.95	.51
41–45							.88	2.20		
46–50							1.75	2.20		
51–55							1.75	.55	.48	
56–60						1.92	.88	2.20		
61–65						1.92	1.75	.55		
66–70							.88	.55		
71–75									.48	
76–80										.51
81–85							1.75			
86–90										
91–95								.55		
96–100										
101–105										
121–125										
126–150					1.19					

Length of pause.	1916–17					Three years combined.				
	November.	December.	January.	February.	March.	November.	December.	January.	February.	March.
Days.	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
2	57.78	48.67	51.16	49.06	64.16	62.05	52.90	52.04	51.59	59.76
3	11.11	13.33	15.61	14.98	12.44	10.26	13.12	14.19	16.25	12.47
4	6.67	8.67	5.65	7.12	7.83	5.64	8.39	5.55	8.04	8.45
5	7.40	3.00	3.65	3.37	4.15	5.13	2.80	3.75	3.02	5.84
2–5	82.96	73.67	76.08	74.53	88.48	83.08	77.20	75.53	78.89	86.52
6–10	6.67	5.00	6.64	8.99	6.91	5.13	4.95	6.20	7.20	7.24
11–15	2.96	2.33	2.99	4.12	2.30	2.56	1.51	3.10	3.35	2.41
16–20	1.48	.66	1.00	2.25	2.30	1.54	.65	1.79	2.18	1.81
21–25	1.48	.66	2.33	3.75		1.54	2.15	2.61	2.85	.60
26–30	1.48	.33	2.66	3.75		2.05	1.08	1.96	3.18	.80
31–35		1.66	3.32	1.50			1.72	3.26	1.17	.20
36–40		.66	1.66	.75		1.03	.65	1.31	.67	
41–45		1.00	.66	.37			.86	.98	.17	
46–50		1.00	1.33				1.08	1.31		
51–55	.74	2.00				.51	1.72	.16	.17	
56–60		1.66	.33			.51	1.29	.82		
61–65		2.00	.33			.51	1.72	.32		
66–70		1.33					1.08	.16		
71–75	.74		.33			.51		.16	.17	.20
76–80		1.00					.65			
81–85		.33					.65			
86–90		.33					.22			
91–95	1.48	1.00				1.03	.65	.16		
96–100										
101–105			.33					.16		
121–125		.33					.23			
126–150										.20

[a] Broody pauses are not included.

Table III gives certain values of interest in connection with Table II. In particular, the ratios of the number of pauses to the number of birds

laying, in spite of marked differences in the value of the ratios from year to year, are in agreement (with one partial exception) in having lower ratios at each end of the season than in the middle.

TABLE III.—*Number of birds laying and number of pauses*

Item.	1913–14					1915–16					1916–17				
	November.	December.	January.	February.	March.	November.	December.	January.	February.	March.	November.	December.	January.	February.	March.
Number of birds laying	23	104	161	176	[a]83	60	128	179	209	220	145	266	303	352	376
Total number of pauses	8	51	130	120	83	52	114	182	210	196	135	300	301	267	217
Ratio of pauses to number of birds laying (expressed in percentage)	34.7	49.0	81.0	68.2	100	86.6	89.0	101.7	100.0	89.1	93.1	112.7	99.3	75.9	57.7
Number without any pause	15	57	83	95	35	34	61	75	88	112	61	89	133	172	213
Percentage without any pause	65.22	54.81	51.55	53.98	42.17	56.67	47.66	41.90	42.11	50.91	42.07	33.46	43.89	48.86	56.65
Number with one or more pauses	8	47	78	81	48	26	67	104	121	108	84	177	170	180	163
Percentage with one or more pauses	34.78	45.19	48.45	46.02	57.83	43.33	52.34	58.10	57.89	49.09	57.93	66.54	56.11	51.14	43.35

[a] A part of this flock was not trap-nested after March 1.

SIGNIFICANCE OF THE PAUSES

It appears from a consideration of the data presented in the preceding paragraphs that pauses differ in their significance. It seems clear that three classes at least can be distinguished: First, the long pause that is clearly indicative of the presence of a winter cycle. The actual length of this pause in exceptional instances need not exceed three or four days, provided it comes at the proper season of the year, and is preceded by a considerable period of egg production. Ordinarily, however, it exceeds 10 days in length. Second, short pauses which occur at frequent intervals in the records of particular individuals because of their very number must be regarded as having considerable significance (see Table I, sire 6781). This type may be called "multipause" provisionally and is to be distinguished from the type in which production is essentially continuous, even though no sharp dividing line can be drawn between these two groups. It is possible that certain records of the multipause type represent a particular genotype, since there is a pronounced tendency for many multipause records to occur in the same families. This tendency has been particularly marked in a small flock of Brown Leghorns. Another multipause type results from intermittent egg production during the winter pause.

A third class includes those individuals which exhibit only one or two short pauses. Some individual records probably represent extreme variants of the multipause type, others pauses at the end of the winter cycle, but the majority are clearly without particular significance.

NUMBER OF PAUSES PER INDIVIDUAL

In Table IV is shown the number of pauses above two days in length per individual, for each of the three years. The data for each of the three years are divided into three groups, determined by the time at which egg production began. The chief points of interest are: (1) the large percentage of records without a pause even when production begins before January, (2) the progressive diminution in number of individuals as the number of pauses rises, and (3) the agreement with expectation—viz, that those birds that begin to lay early should have more pauses than those beginning to lay later in the season.

TABLE IV.—*Number of pauses per individual (disregarding 1- and 2-day pauses), arranged in three periods, according to the time egg production began* [a]

Number of pauses.	Number of individual pauses in—														
	1913–14					1915–16					1916–17				
	Where production began—			Total.	Per cent.	Where production began—			Total.	Per cent.	Where production began—			Total.	Per cent.
	Before January.	During January.	During February.			Before January.	During January.	During February.			Before January.	During January.	During February.		
0	59	29	12	90	47.87	33	32	21	86	32.45	66	26	20	112	27.72
1	34	17	3	54	28.72	61	16	16	93	35.09	111	19	6	136	33.66
2	19	9	1	29	15.43	40	11	1	52	19.62	57	13	1	71	17.57
3	4	5	0	9	4.79	20	3	0	23	8.68	38	5	0	43	10.64
4	1	0	1	2	1.06	5	1	0	6	2.26	18	5	0	23	5.69
5	3	1	0	4	2.13	3	1	0	4	1.51	8	1	0	9	2.23
6	....	....	....	....	....	....	....	....	0	....	1	0	0	1	.25
7	....	....	....	....	....	0	1	0	1	.38	5	0	0	5	1.24
8	....	....	....	....	....	....	....	....	....	....	1	0	0	1	.25
9	....	....	....	....	....	....	....	....	....	....	3	0	0	3	.74
Total number of pauses.	....	....	....	98	52.13	....	....	....	179	67.55	....	....	....	292	72.28
Total number of individuals	....	....	....	188	....	....	....	....	265	....	....	....	....	404	......

[a] A few records in which production began very early in the fall have been omitted.

In Table V the number of instances of different lengths of pauses is shown.

TABLE V.—*Number of instances of pauses 3 to 5 days in length, 6 to 10, and 11 up*

Number of pauses.	1913–14					1915–16					1916–17					Total for 3 years.
	A (3–5 days).	B (6–10 days).	A+B.	C (11 days up).	A+B+C.	A (3–5 days).	B (6–10 days).	A+B.	C (11 days up).	A+B+C.	A (3–5 days).	B (6–10 days).	A+B.	C (11 days up).	A+B+C.	
1	36	13	49	28	77	63	24	87	80	167	89	50	139	96	235	479
2	13	1	14	3	17	19	5	24	18	42	39	11	50	36	86	145
3	4	0	4	0	4	6	1	7	2	9	15	1	16	9	25	38
4	5	0	5	0	5	2	0	2	0	2	10	0	10	0	10	17
5	....	....	....	....	.....	1	0	1	0	1	4	0	4	0	4	5
6	....	....	....	....	.....	....	....	....	....	....	....	....	....	....	....	0
7	....	....	....	....	.....	....	....	....	....	....	....	....	....	....	....	0
8	....	....	....	....	.....	....	....	....	....	....	1	0	1	0	1	1
Total	58	14	72	31	103	91	30	121	100	221	158	62	220	141	361	685

TOTAL LENGTH OF TIME SPENT IN PAUSES

In Table VI the percentage of the flock spending the specified number of days in pauses is given. It should be noted that pauses of one or two days in length have been disregarded in compiling this table. Thus, the row marked zero does not mean that the birds laid continuously or that they had no 2-day pauses, but that the total time spent in pauses, disregarding those of one or two days, was nothing. The same is true for all the other values for the column headed "Total time spent in pauses." An examination of the individual records shows that the 2-day pauses, which might be taken as the lower limit instead of 3-day pauses, are distributed proportionately.

TABLE VI.—*Percentage distribution of the total time spent in pauses*

[Records beginning after January 1 are excluded]

Total time spent in pauses.	Percentage of flock pausing.					
	1913–14		1915–16		1916–17	
	March hatched.	April and May hatched.	March hatched.	April and May hatched.	March hatched.	April and May hatched.
Days.						
0	60.87	53.331	16.22	30.88	15.38	34.08
3–7	13.04	16.67	10.81	11.76	7.69	17.49
8–12	8.70	10.00	4.05	5.88	2.56	4.93
13–17	4.35	1.67	4.05	8.82	....	4.93
18–22	2.17	6.67	5.41	5.88	2.56	2.69
23–27	....	1.67	1.35	5.88	2.56	4.93
28–32	6.52	3.33	....	5.88	2.56	3.59
33–37	2.17	1.67	8.11	7.35	....	4.93
38–42	2.17	....	1.35	2.94	2.56	3.14
43–47	....	1.67	8.11	2.94	2.56	3.14
48–52	....	....	6.76	1.47	7.69	2.24
53–57	....	3.33	2.70	....	2.56	1.79
58–62	....	....	5.41	4.41	2.56	3.14
63–67	....	....	5.41	2.94	7.69	3.14
68–72	....	....	1.35	....	5.13	2.69
73–77	....	....	2.70	....	2.56	....
78–82	....	....	1.35	....	10.26	.45
83–87	....	....	5.41	....	2.56	....
88–92	....	....	....	1.47	2.56	.90
93–97	....	....	1.35	1.47	7.69	.90
98–102	....	....	....	....	2.56	....
103–107	....	....	1.35	....	....	.45
108–112	....	....	1.35	....	....	....
113–117	....	....	....	....	....	....
118–122	....	....	1.35	....	....	....
123–127	....	....	....	....	2.56	....
128–132	....	....	1.35	....	2.56	.45
133–137	....	....	1.35	....	....	....
138–142	....	....	....	....	2.56	....
143–147	....	....	1.35	....	....	....

Several features of this table require comment. First, the large percentage of the 1913-14 flock that falls in the zero class, and the low value of the pause of maximum length. It should be noted that this flock was not put into the laying houses until late October, and none of them began to lay until after November 1. Second, the high percentage of the flocks of the April and May hatches of 1915–16 and 1916–17 that spent practically no time at all in pauses. Thus, the sums of the first two rows are 42.64 and 51.56 per cent, respectively. This high value is the more remarkable for the season of 1916–17, since the birds began to lay early in the fall. Contrasted with this is the third point—viz, the relatively low percentage of the March-hatched pullets of these two years that fall into these rows. This may perhaps be explained by the tendency of March-hatched pullets to begin to lay early in the fall and to undergo a winter molt.[1] That the 1913–14 flocks shows a radically different distribution is probably due to its delay in beginning production. This raises the question as to whether or not the time a bird begins to lay may not have a considerable effect on the appearance or nonappearance of the winter pause. The individual records, however, prove that no necessary relation of the sort postulated exists.

B.—RATE OF PRODUCTION

VALUE OF MONTHLY EGG PRODUCTION AS AN INDEX OF A WINTER CYCLE

A cycle in egg production may be indicated by a lessened daily rate of production as well as by a pause. Specifically one would expect of the winter cycle, if it were delineated only by a change in rate, either that production should begin at a relatively high rate to be followed during the latter part of the winter by a period of lessened production or that the production should start at a slow rate, rise to a maximum, and then decline. Following Pearl, we should anticipate that February would have less eggs than January.

In handling the data given in Tables VII to XI we have proceeded as follows, unless otherwise specified. Each lot of Rhode Island Reds is a group of individuals selected for the following reasons: (1) A laying period of considerable length in order that ample time should be allowed for the completion of the entire cycle—that is, both egg production and pause. Thus, birds beginning to lay after December 1 are excluded from the tables. (2) Records containing broody pauses are excluded. (3) Each month should be equal in length. We utilize, therefore, three periods of 31 days each—viz, the months of December, January, and February, including in the latter the first three days of March. (4) The data, furthermore, are divided into two groups—viz, March-hatched

[1] Possibly the large percentage of March-hatched pullets with pauses may be interpreted as being due to a greater opportunity for the appearance of the winter cycle, since they begin to lay early in the season. Pearl, however, does not mention an association of a molt with the winter pause.

pullets and those hatched in April and May, the hatching date of the latter being the same as for the Maine birds.

The data for Barred Plymouth Rocks and Wyandottes have been extracted from the Maine Station Bulletins 79 and 93 (*3, 4*) and have been handled in essentially the same way. As these records have been given for months only, only those birds that laid at least one egg in November have been included in the tables. Dr. Pearl has stated to the writer that his Barred Plymouth Rocks do not go broody during the winter, so that we have assumed that broodiness does not enter as a disturbing factor. The February records have been corrected to a basis of 31 days.

TABLE VII.—*Mean monthly egg production of three breeds of pullets beginning to lay before December 1*

[Broody records are excluded. No record is included where production began later than December 1. No Barred Plymouth Rocks or White Wyandottes that failed to lay in November are included. February records are reduced to a 31-day basis]

Flock of—	Hatched.	Number of individuals.	December.	January.	February.
BARRED PLYMOUTH ROCKS					
1899–1900		38	17.3	19.4	13.4
1900–1901		33	16.2	15.9	17.6
1901–1902		25	17.2	17.2	11.2
Total		96	16.9	17.6	14.3
WHITE WYANDOTTES					
1899–1900		34	18.8	17.4	14.7
1900–1901		58	12.7	16.9	13.4
1901–1902		11	15.8	13.2	12.7
Total		103	15.0	16.7	13.8
RHODE ISLAND REDS					
1913–14	March	10	21.1	21.5	20.6
	April and May	13	21.2	21.9	21.6
1915–16	March	56	17.0	14.0	12.9
	April and May	24	19.9	14.9	12.8
1916–17	March	32	11.6	7.9	12.9
	April and May	122	19.3	15.0	16.3
Total		257	18.1	14.5	15.2

From Table VII it is clear that the egg production of February is usually lower than that for January. Some exceptions are to be noted, especially in the Rhode Island Reds, and also for 1900–1901 in the Barred Plymouth

Rocks. The Rhode Island Reds, compared with the Barred Plymouth Rocks and the White Wyandottes, show a less difference between the two months. In this connection it may be noted that the mean value of the February egg production for eight years, as given for the Barred Plymouth Rocks by Pearl (7) if reduced to a basis of 31 days is 12.03, approximately, or slightly higher than for January, which is 11.71. The mean egg production for February (28 days) for the entire flock of our Rhode Island Reds has always been higher than for January.

Table VIII is similar to Table VII, but we have utilized the larger numbers made available by including in the January and February means the records made by birds beginning to lay as late as January 1. The essential result is the same as shown in Table VII.

TABLE VIII.—*Mean monthly egg production of pullets from the same flocks as Table VII, but including those beginning to lay as late as January 1*

BARRED PLYMOUTH ROCKS

Flock of—	Hatched.	Number of individuals laying in— December.	Number of individuals laying in— January and February.	December.	January.	February.
1899–1900		38	55	17.3	18.7	11.8
1900–1901		33	56	16.2	15.5	16.5
1901–1902		25	45	17.2	15.4	12.4
Total		96	156	16.9	16.6	13.7

WHITE WYANDOTTES

Flock of—	Hatched.	Number of individuals laying in— December.	Number of individuals laying in— January and February.	December.	January.	February.
1899–1900		34	50	18.8	18.3	14.0
1900–1901		58	77	12.7	16.9	14.6
1901–1902		11	31	15.8	16.5	12.7
Total		103	158	15.0	17.2	14.0

RHODE ISLAND REDS

Flock of—	Hatched.	Number of individuals laying in— December.	Number of individuals laying in— January and February.	December.	January.	February.
1913–14	March	10	46	21.1	20.5	19.7
	April and May	13	62	21.2	20.6	19.9
1915–16	March	56	90	19.9	14.3	13.5
	April and May	24	70	17.1	16.8	15.3
1916–17	March	32	44	11.6	8.3	11.1
	April and May	122	230	19.3	16.1	17.0
Total		256	542	17.7	14.8	16.0

For the Barred Plymouth Rocks and White Wyandottes the December production varies in the individual years, being sometimes greater,

sometimes less than for January. For the Rhode Island Reds the December production is considerably greater than for January and also for February.[1]

TABLE IX.—*Mean monthly egg production for all Rhode Island Reds that began to lay by December 1, separated into two groups, according to length of pauses*

[Any record having a single pause of more than 10 days in length is included in the group of pauses of 11 days or more. A record having several short pauses of less than 11 days each, even though aggregating more than 10 days, is included in the groups of pauses of less than 11 days]

Flock of—	Grouping of records.	Hatched.	Number of individuals.	December.	January.	February (31 days).
1913–14	All birds.		23	21.1	21.7	20.9
	Records with pauses not exceeding 10 days in length.	March	9	21.0	22.0	22.2
		April–May	11	22.6	23.1	22.3
		Hatches combined	20	21.9	22.6	22.3
	Records with pauses of 11 days or more.	March	1	22.0	17.0	1.1
		April–May	2	13.0	15.5	16.5
		Hatches combined	3	16.0	16.0	11.4
1915–16	All birds.		80	17.9	14.3	12.9
	Records with pauses not exceeding 11 days in length.	March	20	19.4	19.9	20.0
		April–May	11	19.5	19.2	18.4
		Hatches combined	31	19.5	19.6	19.4
	Records with pauses of 11 days or more.	March	36	15.7	10.8	9.0
		April–May	13	20.3	11.2	8.0
		Hatches combined	49	16.9	10.9	8.7
1916–17	All birds.		154	17.8	13.6	15.5
	Records with pauses not exceeding 11 days in length.	March	7	21.7	19.7	20.7
		April–May	69	22.4	21.1	20.9
		Hatches combined	76	21.7	19.7	20.7
	Records with pauses of 11 days or more.	March	25	8.8	4.6	8.8
		April–May	53	15.6	7.2	10.7
		Hatches combined	78	13.4	6.1	10.1

If the data for Rhode Island Reds are analyzed further, as is done in Table IX, certain interesting facts come to light. In this table the records have been divided into two groups, one consisting of those records

[1] A comparison was attempted between November and December egg production, but no satisfactory results could be obtained, because of the small number of birds which had complete records for November.

that have no pauses exceeding 10 days in length, and the second of those that have pauses of more than 10 days. In the former group the egg production remains at essentially the same point in all three months. In the second group, however, the January and February production is markedly lower than that of December. The production for January may or may not exceed that of February.[1]

DIFFERENCES IN MONTHLY EGG PRODUCTION DURING DECEMBER, JANUARY, AND FEBRUARY FOR RHODE ISLAND REDS, BARRED PLYMOUTH ROCKS, AND WHITE WYANDOTTES

In Table X we have examined the differences in the monthly egg production for the same set of birds shown in Tables VII and VIII. For the Barred Plymouth Rocks and White Wyandottes it is noticeable that for each year but one the number of birds laying more eggs in January than February is greater than the number laying more eggs in February than January (in the exception the number is equal) and that in five out of six instances the average number of eggs laid by the birds having an excess January production is greater than for those having an excess production in February. The percentage of birds having an excess January production is 75.29 for Barred Plymouth Rocks and 68.23 for the White Wyandottes.

The data for the Rhode Island Reds are quite different in character. Only 51.39 per cent of the March-hatched pullets lay more eggs in January than February, while for April and May hatched pullets the value is 47.16 per cent, although in two years out of three the number of birds with a January excess is greater than for February. In only one instance, and that with a small number of birds involved, is the number much more than twice the number having an excess February production. This is to be compared with the Maine records, where in every instance the number of birds with a January excess is greater than those with a February excess and, except in two instances, are two to three times as numerous (in one case 16 times). For the Rhode Island Reds hatched in April and May the average number of excess eggs in two years out of three is greater for those pullets with an excess February production. For the March-hatched pullets conditions are reversed.

[1] It is possible that the two groups, high producers and mediocre producers, should be separated in an analysis of this sort. So few of the latter were available, however, that it seemed unwise to attempt the separation.

TABLE X.—*Differences in mean production for the months of December and January, December and February, and January and February for the several flocks studied. All birds began egg production on or before December 1*

RHODE ISLAND REDS

Flock of—	Month hatched.	No difference.	December greater than January.			December less than January.			No difference.	December greater than February.			December less than February.			No difference.	January greater than February.			January less than February.		
			Number of individuals.	Total excess.	Mean excess.	Number of individuals.	Total deficiency.	Mean deficiency.		Number of individuals.	Total excess.	Mean excess.	Number of individuals.	Total deficiency.	Mean deficiency.		Number of individuals.	Total excess.	Mean excess.	Number of individuals.	Total deficiency.	Mean deficiency.
1913–14	March	0	4	10	2.50	6	21	3.50	3	2	8	4.00	5	15	3.00	3	4	14	3.50	3	10	3.33
	April and May	2	4	10	2.50	5	15	3.00	2	6	18	3.00	3	14	4.66	5	5	12	2.40	1	3	3.00
	Total	2	8	20	2.50	11	36	3.27	5	8	26	3.25	8	29	3.62	8	9	26	2.88	4	13	3.25
1915–16	March	2	30	205	6.83	19	69	3.63	2	31	319	10.29	18	100	5.50	5	26	206	7.92	20	121	6.05
	April	1	21	166	7.90	3	19	6.33	1	20	211	10.55	4	24	6.00	0	15	101	6.73	10	51	5.10
	Total	3	51	371	7.27	22	88	4.00	3	51	530	10.39	22	124	5.63	5	41	307	7.48	30	172	5.73
1916–17	March	1	16	106	6.62	4	20	5.00	0	11	114	10.36	10	84	8.40	2	7	61	8.71	12	117	9.75
	April and May	9	84	560	6.66	26	91	3.50	6	81	483	5.96	32	155	4.84	10	48	224	4.66	61	365	5.98
	Total	10	100	666	6.66	30	111	3.70	6	92	597	6.48	42	239	5.69	12	55	285	5.18	73	482	6.60
	Total	30	159	1,057	6.64	63	235	3.73	28	151	1,153	7.63	72	392	5.44	50	105	618	5.88	107	667	6.23
	Per cent	......	71.62	......	......	28.37	......	......	......	67.71	......	......	32.28	......	......	......	49.52	......	......	50.47	......	......

BARRED PLYMOUTH ROCKS

Flock of—	Month hatched.	No difference.	December greater than January.			December less than January.			No difference.	December greater than February.			December less than February.			No difference.	January greater than February.			January less than February.		
			Number of individuals.	Total excess.	Mean excess.	Number of individuals.	Total deficiency.	Mean deficiency.		Number of individuals.	Total excess.	Mean excess.	Number of individuals.	Total deficiency.	Mean deficiency.		Number of individuals.	Total excess.	Mean excess.	Number of individuals.	Total deficiency.	Mean deficiency.
1899–1900	April and May	2	12	61	5.08	21	133	6.33	1	24	203	8.45	10	60	6.00	1	32	222	6.93	2	7	3.50
1900–1901	April and May	2	15	105	7.00	14	85	6.07	4	14	71	5.07	13	102	7.84	2	15	76	5.06	14	127	9.07
1901–1902	April and May	2	13	70	5.38	8	61	7.62	2	16	155	9.68	5	18	3.60	1	17	149	8.76	5	21	4.20
	Total	6	40	236	5.90	43	279	6.48	7	54	429	7.94	28	180	6.42	4	64	447	6.98	21	155	7.38
	Per cent	......	48.19	......	......	51.80	......	......	......	65.85	......	......	34.14	......	......	......	75.29	......	......	24.70	......	......

WHITE WYANDOTTES

1899–1900	April and May	5	16	113	7.06	11	51	4.64	2	23	189	8.21	7	50	7.14	3	22	130	5.90	7	52	7.42
1900–1901	April and May	4	14	82	5.85	34	289	8.50	4	22	136	6.18	26	216	8.30	4	32	205	6.40	16	78	4.87
1901–1902	April and May	1	6	42	7.00	1	3	3.00	0	5	55	11.00	3	9	3.00	0	4	34	8.50	4	27	6.75
	Total	10	36	237	6.58	46	343	7.45	6	50	380	7.60	36	275	7.63	7	58	369	6.36	27	157	5.81
	Per cent	......	43.90	......	......	56.09	......	......	......	58.13	......	......	41.86	......	......	......	68.23	......	......	31.76	......	......

A consideration of the number of individuals laying an excess number of eggs for December over January (and also February) shows that more of the Rhode Island Reds have a December excess than a January (or February) excess. For the Barred Plymouth Rocks and White Wyandottes more individuals have a January production in excess of that of December, but a February deficiency. The difference in the case of the Barred Plymouth Rocks, however, is not great. Nevertheless it is clear that the egg production of these two varieties does not undergo a period of depression at the same season as that of the Rhode Island Reds.

TABLE XI.—*Distribution of individuals having a greater egg production in January over February, or vice versa, arranged according to the maximum length of any single pause*

	January production greater than February.									February production greater than January.								
	1913–14			1915–16			1916–17			1913–14			1915–16			1916–17		
Length of pauses..days..	0	3–10	11–up.	0	3–10	11–up.	0	3–10	11–up.	0	3–10	11–up.	0	3–10	11–up.	0	3–10	11–up.
EXCESS NUMBER OF EGGS.																		
1	11	....	....	4	2	2	15	6	5	7	3	2	5	2	2	11	10	3
2	8	1	....	1	4	1	6	6	3	5	1	1	2	2	2	11	7	5
3	4	2	....	3	3	2	7	7	1	5	2	2	6	3	3	8	9	1
4	1	4	....	....	2	3	3	3	2	5	3	....	3	3	1	....	3	8
5	....	2	1	....	2	1	2	1	2	....	3	1	1	2	2	....	3	9
6	....	....	....	1	....	3	1	2	3	....	....	1	....	....	1	1	....	1
7	....	1	1	....	1	6	....	....	3	....	....	....	....	....	2	1	2	5
8	....	....	....	....	2	2	....	....	1	....	....	1	....	....	3	....	....	1
9	....	1	....	....	1	2	....	....	1	....	....	....	....	....	2	....	2	2
10	....	....	....	....	....	3	....	1	....	....	....	....	....	....	1	....	....	1
11	....	....	1	....	....	3	....	....	4	....	....	....	....	....	....	....	....	3
12	....	....	....	....	....	3	....	....	1	....	....	....	....	....	....	....	....	5
13	....	....	2	....	....	3	....	....	4	....	....	2	....	....	1	....	....	4
14	....	....	1	....	....	4	....	....	1	....	....	....	....	....	2	....	....	1
15	....	....	....	....	....	3	....	....	2	....	....	....	....	....	1	....	....	5
16	....	....	2	....	....	2	....	....	1	....	....	....	....	....	....	....	....	1
17	....	....	....	....	....	....	....	....	1	....	....	....	....	....	....	....	....	3
18	....	....	....	....	....	....	....	....	2	....	....	....	....	....	3	....	....	1
19	....	....	1	....	....	....	....	....	1	....	....	....	....	....	1	....	....	1
20	....	....	....	....	....	1	....	....	1	....	....	....	....	....	1	....	....	2
21	....	....	....	....	....	....	....	....	....	....	....	....	....	....	1	....	....	1
22	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
23	....	....	....	....	....	....	....	....	1	....	....	....	....	....	1	....	....	....
Total	24	11	9	9	17	44	34	26	40	22	12	10	17	12	30	32	36	63
Sum of first two columns in each section	....	35	....	....	26	....	....	60	....	....	34	....	....	29	....	....	68	....
Number with the same production in each month	15	5	0	6	0	4	18	6	9	....	....	....	....	....	....	....	....	....

SUMMARY

	Production equal.			February production greater than January.			January production greater than February.		
Length of pause................days..	0	3–10	11–up.	0	3–10	11–up.	0	3–10	11–up.
Number of individuals	39	11	13	71	60	103	67	54	93
Sum of first two columns in each section	......	50	......	......	131	......	......	121	......
Sum of all three columns in each section	......	......	63	......	......	234	......	......	214

DIFFERENCE IN JANUARY AND FEBRUARY EGG PRODUCTION, TAKING THE LENGTH OF PAUSE INTO CONSIDERATION

The results of an examination of this point are shown in Table XI, which gives no evidence that a difference in the egg production of an individual for the two months is more likely to occur in one month than the other, since the number of individuals falling in the different categories is approximately equal, although fluctuating somewhat from year to year.

These data, taken in connection with those previously discussed regarding differences in monthly production and with the observations on rate of production, show that a lower rate of production for February over January in the individual case is without significance, unless associated with a definite pause. This information is of particular value for those instances in which the production for February is only a few eggs less than for January and which, from Pearl's data, might be considered to exhibit a winter cycle.

TABLE XII.—*Mean production of two groups of pullets hatched in April, 1916, for the periods after the first egg designated in the headings*

BIRDS BEGINNING TO LAY OCT. 5–DEC. 13, INCLUSIVE (80 TO 72 INDIVIDUALS[a])

Days of production	1–31	32–62	63–93	1–10	11–41	42–72	73–103	Mean March production.
Mean number of eggs per period	16.96	16.60	14.56	4.93	17.63	15.87	15.01	20.81
Mean date of beginning and end of each period	Nov. 9 to Dec. 10.	Dec. 11 to Jan. 10.	Jan. 11 to Feb. 10.	Nov. 9 to Nov. 19.	Nov. 20 to Dec. 20.	Dec. 21 to Jan. 20.	Jan. 21 to Feb. 20.	

BIRDS BEGINNING TO LAY DEC. 14–JAN. 25, INCLUSIVE (69 TO 55 INDIVIDUALS[a])

Mean number of eggs per period	17.11	17.92	21.68	5.24	17.50	19.28	22.03	
Mean date of beginning and end of each period	Jan. 2 to Feb. 1.	Feb. 2 to Mar. 3.	Mar. 4 to Apr. 4.	Jan. 2 to Jan. 12.	Jan. 13 to Feb. 12.	Feb. 13 to Mar. 16.	Mar. 17 to Apr. 16.	

[a] The variation number of individuals is due to broodiness.

EGG PRODUCTION IN PERIODS OF DEFINITE LENGTH, BEGINNING WITH FIRST EGG

In the preceding paragraphs we have used records that begin before a definite date—viz, December 1. On being examined from the standpoint of the first egg it will be observed that the production for any one month, say December, is made up of the production of birds that have been laying for varying intervals of time, including those well along in production and those just beginning. In Table XII the rate of production has been examined from another standpoint. Three periods of

31 days each, beginning with the date of the first egg, and three periods of 31 days each beginning with the eleventh day of production have been employed in studying the production of two groups of April-hatched pullets. One group has a mean date of first egg on November 9; the other, on January 2. The reason for employing two sets of three periods of 31 days, each differing by 10 days, for each lot, lies in the fact that egg production sometimes is extremely slow and erratic at the start and that this may reduce the egg production disproportionately for the first 31-day period.

The following points are shown by Table XII: First, the egg production of the first group is somewhat inferior to that of the second. Note particularly the March production of the first group compared with that period of the second that extends from March 4 to April 4. Second, while there is a fall from the first 31-day period through each of the two successive periods in the first group until March, the second group shows a constant rise from period to period, which may mean that the time of year in which the various periods fall is concerned with the drop in production, for it will be noted that the first period of the second group nearly coincides with the third period of the first group.

CONCLUSIONS REGARDING THE CRITERIA FOR THE WINTER CYCLE IN THE INDIVIDUAL

A consideration of the data presented in the preceding pages leads to the following conclusions regarding criteria by which the winter cycle can be recognized in the individual record.

First. The rate of production, as shown by the monthly egg records, fails to furnish a satisfactory index of the existence of a winter cycle in the individual Rhode Island Red pullet.

Second. The best criterion of the existence of a winter cycle in the individual is the existence of a pause in production beginning in December, January, February, or, rarely, March, following a period of continuous egg production, and usually exceeding 10 days in length. A single pause in some instances may be replaced by series of short pauses separated by only one or two eggs.[1]

Third. In some instances a short pause—that is, 10 days or less in length—occurring in February or March and following a period of several weeks of continuous egg production may delimit the winter cycle.

It seems clear that the period of low flock production for the Rhode Island Reds, for birds beginning to lay sufficiently early in the season may come earlier in the winter than at the Maine Station. In some

[1] The second part of the two recent bulletins from the Utah Station—viz, Ball, Alder, and Egbert (*1*), and Ball and Alder (*2*)—was received after the manuscript of this paper had been completed. Only a very brief comment can be made on their discussion of the "'winter' egg-laying period" in White Leghorns. They conclude "that there is no apparent biological ground for either the beginning or end of this period . . ." This conclusion, which rests on mass statistics, needs reexamination before it can be considered of universal applicability to all White Leghorns.

individuals the pause comes comparatively early in the winter so that the following [spring (?) cf. Pearl and Surface (7)] cycle of production may begin as early as the middle of January. This may mean, perhaps, that the winter pause in Rhode Island Reds is not homologous with that of the Maine Station birds. Whether or not this is so, it would seem desirable to look at it from a somewhat different standpoint. It may be that this pause follows an initial cycle of production. It may be, too, that we are not dealing wholly with an inherent pause but with a pause that depends in part on the environment for its manifestation, which is due to a difference in resistance on the part of individuals to the weather conditions at this season of the year.

NUMBER OF EGGS LAID BEFORE THE WINTER PAUSE AND LENGTH OF WINTER PAUSE

In those instances where the winter pauses could be determined with some degree of accuracy, we have determined the range and mean number of eggs laid before the pause and the same constants for the pause itself, for the pullets of the April and May hatches shown in Table XIII. It should be borne in mind, however, that in most instances the limit specified earlier has been used for the lowest number of days indicative of the winter pause.

For the number of eggs before the pause, the range has a value of 2 to 96,[1] with a mean of 35.98 eggs. The length of the pause has a range of 8 to 72 days, with a mean of 34.23 days. For the birds listed under section B of Table XIII, the values are for eggs; range 1 to 96,[1] mean, 36.98, and for length of pause range, 3 to 104, mean 24.87.

The possibility of a correlation between the number of eggs laid before the pause and the length of the pause has been examined and found to be practically nonexistent.

MODE OF INHERITANCE OF THE WINTER CYCLE

From the data that have been presented in the preceding pages it is clear that some individual Rhode Island Reds exhibit a definite winter cycle, while others as definitely show no winter cycle. For purposes of description we may describe the former as "winter cycle," the latter as "no winter cycle." It is clear, moreover, from an examination of the family records (Tables I and XIII) that the character is inherited and that segregation takes place. When the ratios are examined, however, no evidence of an entirely satisfactory character is afforded us as to the mode of inheritance of the winter cycle, although there is some evidence that the winter cycle is inherited according to the simple

[1] The rather absurd values for the lower end of the range result from the inclusion of a few records that are obviously out of the ordinary but which can not be excluded. The next lower value is 9.

Mendelian scheme, "no winter cycle" being the dominant allelomorph, "winter cycle" the recessive.

Before considering the evidence for this scheme there are certain peculiarities of the character that should be listed.

First. The character does not come to visible expression in the male; hence, his gametic composition can be made out only through a consideration of his maternal ancestors, his daughters, and his sisters.

Second. The character is a physiological one, subject to possible influences by the environment, and perhaps to other internal factors concerned with egg production.

Third. Pauses distinct from those indicative of the winter cycle but likely to be mistaken for them may occur.

Fourth. Difficulties are encountered in classifying certain individual records, such as records with several very short pauses, a single very short pause, a simple slackening in rate of production without any definite pause, and pauses occurring at the limits of the season. In such instances we have proceeded somewhat arbitrarily. There have been excluded, first, all birds that have laid less than 20 eggs after the first of January; second, all birds showing broody pauses; third, March-hatched birds that begin to lay early in the season, and which molt.

TABLE XIII.—*Progeny of individual pairs of birds of flock of 1916–17, showing the number with a winter cycle (P), those without (N), and those on which a definite determination could not be made (X)*

Father.	Mother.	Presence or absence of winter cycle in mother's record as determined by two methods.	Method A.				Method B.			
			P	N	X	Total.	P	N	X	Total.
4723	4473	N X......	6	5	12	23	12	4	7	23
	5476	N P......	1	9	0	10	2	8	0	10
	5463	N P......	2	3	2	7	3	3	1	7
	4178	N N......	3	3	4	10	7	1	2	10
	4845	P P......	3	8	1	12	5	6	1	12
	4138	P P......	4	6	5	15	5	6	4	15
5581	4553	X P......	0	1	6	7	0	1	6	7
	4533	X P......	0	5	0	5	0	5	0	5
	5895	X P......	2	3	2	7	2	3	2	7
	2564	X P......	3	3	5	11	6	2	3	11
	5771	X P......	2	4	2	8	4	3	1	8
	4529	X X......	0	0	6	6	3	0	3	6
	2565	X X......	1	0	2	3	1	0	2	3
5077	5703	X P......	1	2	1	4	1	2	1	4
	6840	N N......	0	0	4	4	0	1	3	4
	6767	N P......	2	3	0	5	3	2	0	5
6265	5719	N P......	1	4	7	12	4	4	4	12
	5159	X P......	1	4	1	6	1	4	1	6
	5982	N N......	0	4	3	7	3	2	2	7
4354	4518	P P......	1	3	4	8	3	3	2	8
	5412	X P......	1	7	1	9	3	6	0	9
	4828	X P......	2	6	2	10	5	4	1	10
	5090	X P......	0	8	2	10	3	6	1	10
	4030	N N......	0	0	2	2	0	0	2	2
4312	4254	X P......	0	0	1	1	0	0	1	1
	49	N N......	1	0	7	8	2	0	6	8
6781	4509	P P......	1	8	2	11	5	4	2	11
	3719	N X......	1	2	3	6	2	1	3	6
	3482	N N......	3	7	4	14	6	5	3	14
6373	3600	X X......	2	2	7	11	5	2	4	11
	174	X P......	2	0	2	4	2	0	2	4
	284	X X......	1	1	3	5	1	1	3	5
4378	4043	N N......	2	1	4	7	3	0	4	7
	4754	P P......	0	0	8	8	0	0	8	8
	4012	N N......	4	2	5	11	4	2	5	11
	4592	P P......	1	0	3	4	1	0	3	4
4786	6684	X X......	0	0	2	2	0	0	2	2
	5881	X X......	0	6	2	8	1	5	2	8
3617	4409	P P......	0	4	1	5	3	1	1	5
	4716	N P......	0	8	2	10	1	7	2	10
	5230	N P......	0	2	1	3	1	2	0	3
	6751	N N......	1	5	1	7	1	5	1	7
	6003	N N......	0	9	1	10	3	6	1	10
5477	4844	X X......	2	2	7	11	2	2	7	11
	6366	N N......	1	3	0	4	1	3	0	4
5240	6404	X X......	3	4	0	7	4	3	0	7
5776	6232	P P......	0	5	3	8	2	3	3	8
	5098	N N......	0	0	2	2	2	0	0	2
4882	2532	X P......	0	13	13	26	4	12	10	26
		Total...	61	175	158	394	132	140	122	394

In Table XIII is shown the classification of the flocks of 1916–17, arranged by families. Each individual is classified twice. According to one classification (method A) our endeavor has been to ascertain as closely as our best judgment would permit, the true status of each individual. In method B, however, we have given the benefit of any doubt, to the winter cycle and have listed every bird in the positive column that could possibly be considered as having a winter cycle.

Section A gives the ratio of 61 individuals with a winter cycle to 174 without. This is close to the 1 to 3 ratio—viz, expected 58¾ to 176¼—for a simple Mendelian case of inheritance. According to method B, however, the ratio approaches closely to equality.

Table XIV gives the ratios for the flocks as a whole for 1913–14 and 1915–16, in addition to 1916–17, and the grand total. The years vary somewhat, but the total, 147 to 388, is perhaps merely a deviation from the expected 133¾ to 401¼, the deviation being in the direction expected, on the assumption that birds without the genes for a winter cycle may exhibit a false cycle.

TABLE XIV.—*Number of individuals classified according to the presence or absence of the winter cycle for the years 1913–14, 1915–16, and 1916–17*

Flock of—	Method A.				Method B.			
	Birds with a winter cycle.	Birds without a winter cycle.	Undeterminable.	Total.	Birds with a winter cycle.	Birds without a winter cycle.	Undeterminable.	Total.
1913–14	25	114	85	224	53	99	72	224
1915–16	61	100	139	300	104	79	117	300
1916–17	61	175	158	394	132	140	122	394
Total	147	388	......	......	289	318	......	......
Expected	133¾	401¼	......	......	(?)	(?)	......	......

For method B the observed ratio for the three seasons is 289 to 318.

Mass figures of the sort just given are merely suggestive, since the ratio 1 to 3 holds only under certain conditions. However, an examination of the proportions in which the two types occur among the progeny of a single female, and in some instances of the progeny of one male by several females, favors the suggestion given above. At the same time, the gametic constitution of the parents can not be made out with a satisfactory degree of accuracy. To be sure, one can assign a gametic constitution to many individuals, but it is impossible to check these by reference to preceding years, mainly because the number of progeny from a single pair in the earlier years was too small to afford critical evidence. Since prospective matings are likely to furnish critical evidence on the point in question, it seems advisable to defer any attempt at a solution of this phase of the problem for the present.

SUMMARY

(1) An examination of the data published by Gowell confirms the statements of Pearl and Surface (7) and Pearl (5, 6) regarding the presence of a winter cycle in Barred Plymouth Rocks.

(2) The winter cycle is much more characteristic of the Maine flocks as a whole than it is of our Rhode Island Reds, where it can be demonstrated in only a portion of the flock.

(3) The period of decreased flock egg production for Barred Plymouth Rocks and White Wyandottes comes in February. For Rhode Island Reds it may come in January as well as in February.

(4) A pause, or series of pauses, usually exceeding 10 days in length and following a considerable period of regular egg production, is the best index of the existence of a winter cycle in the individual Rhode Island Red.

(5) The rate of production does not furnish a satisfactory index of the presence or absence of a winter cycle.

(6) Evidence is presented which indicates that the winter cycle may be inherited in some definite but undetermined manner.

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DIGESTION OF STARCH BY THE YOUNG CALF

By R. H. Shaw, T. E. Woodward, and R. P. Norton, *of the Dairy Division, Bureau of Animal Industry, United States Department of Agriculture*

PREVIOUS INVESTIGATIONS

There is considerable evidence that young animals thrive on a ration containing starch, but a rather extensive search in the literature failed to discover any data concerning the question as to how soon after birth the calf can begin to digest starch. The investigation here described was undertaken for the purpose of ascertaining how early in its life the calf can utilize starch or starch-containing feeds. The practical application, of course, is in supplementing or supplanting the milk ration of the young calf with other feed.

The literature contains many accounts of feeding experiments with young animals where starch alone or as the principal component of some feed has been used. The purposes of these experiments, however, have been largely to determine the effect of starch upon the health, the rate of gain in weight, the cost of raising, or the digestibility of some other component of the ration rather than the actual digestibility of the starch itself. The record of but one experiment was found in which the feces of young starch-fed calves were tested for the presence of starch.

Ewing and Wells [1] report the use of starch in combination with corn silage and cottonseed meal in the ration of 12-month-old steers on digestion trial. In their summary they state that when as much as 47.3 per cent of the net energy of the ration was supplied in the form of starch the iodin test did not indicate the presence of starch in the feces.

There are recorded in the medical literature on the diet and hygiene of children several investigations in which the actual digestibility of starch by children was studied. Kerley, Campbell, and Mason [2] report the examination for starch of 324 stools, collected under controlled conditions at the New York Infant Asylum from 60 children, all under 1 year of age, who had been fed either wholly or in part on barley water. The barley water was prepared by boiling raw barley flour for 1½ hours. The stools were examined for starch by the Von Jaksch iodin test, with

[1] Ewing, Perry van, and Wells, C. A. THE ASSOCIATIVE DIGESTIBILITY OF CORN SILAGE, COTTONSEED MEAL, AND STARCH IN STEER RATIONS. Ga. Agr. Exp. Sta. Bul. 115, p. 269–296, 7 diagr., 1915.

[2] Kerley, C. G., Campbell, W. C., and Mason, H. N. A FURTHER CONTRIBUTION TO THE STUDY OF STOOLS OF STARCH-FED INFANTS. *In* Jour. Amer. Med. Assoc., v. 47, no. 10, p. 763–765. 1906.

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Lugol's solution. Of the 60 children, 33 always gave negative iodin tests, indicating complete utilization of the starch. Among the remainder, 8 usually gave a negative test, 12 usually gave a positive test, and 7 always a positive test for starch. Of the 41 children showing a good capacity for starch utilization one 19-day-old child received 9.2 gm. of barley flour daily for 2 days; one 21-day-old child received 14.6 gm. every 24 hours; one child 1 month and 22 days old received 25.9 gm. the first day and 25.3 gm. a day for the following four days; and another child 1 month and 19 days old received 12 gm. daily for three days.

Heubner,[1] in a paper presented before the Berlin Medical Society, describes an investigation conducted at Leipzig for the purpose of determining the digestibility of starch in the food of artificially fed children. The children received during 1-day and 2-day periods a carefully prepared starchy gruel which was fed in place of milk, at the same intervals and in the same quantity as the milk feeding. Carbon was used to identify the experimental stools, and the feces from each child were assembled, dried, and analyzed for starch. A 7-weeks-old child received 24.6 gm. of rice flour during a 25-hour period, and no starch was found in the feces. Another child 14 weeks old received 53 gm. of rice flour during a 39-hour period, and 0.1689 gm. of starch was found in the feces. A third child 1 year old received, in addition to 72 gm. of butter, 133 gm. of rice flour during a 48-hour period, and 0.2804 gm. of starch was found in the feces. A fourth child, 14 weeks old, received 57 gm. of a specially prepared oatmeal during a 34-hour period, and 0.2611 gm. of starch was found in the feces.

In an elaboration of the work done by Heubner at Leipzig, Carstens[2] gives the results of digestion experiments on eight children from 5 to 14 weeks old. Some of these children received starch from rice flour, some from a prepared oatmeal flour, and some from two different proprietary infant foods. The same methods were followed as in the Heubner investigation. The quantity of undigested starch varied from a trace in the feces of two children, one 9 weeks and one 15 weeks old, respectively, to 5.08 gm., or 6.23 per cent of the amount ingested by a child 6½ weeks old.

Krüger[1] who worked with fetal and newborn calves found that the ptyalin is secreted in the salivary glands as early as the seventh month of fetal life, but that while the quantity increases up to birth, even at that time it is too small to be of any importance in the digestion of food.

[1] HEUBNER, O. UEBER DIE AUSNÜTZUNG DES MEHLS IM DARM JUNGER SÄUGLINGE. *In* Berlin. Klin. Wchnschr., Bd. 32, No. 10, p. 201–204, 1895. Literatur, p. 204.

[2] CARSTENS, J. H. WEITERE ERFAHRUNGEN ÜBER DIE AUSNÜTZUNG DES MEHLS IM DARME JUNGER SÄUGLINGE. *In* Verhandl. Gesell. Kinderheilk., Bd. 12, p. 169–176. 1895.

[1] KRÜGER, Friedrich. DIE VERDAUUNGSFERMENTE BEIM EMBRYO UND NEUGEBORENEN. 80 p. Wiesbaden, 1891. Literatur, p. 79–80.

EXPERIMENTAL WORK

Two male calves, each 4 days old, were selected. Each was fed 5.44 kgm. of whole milk a day in two feedings. Beginning at 4 days of age, each calf received 40 gm. of ordinary cornstarch per feeding, in addition to the milk, for a period of three days. The starch ration was prepared as follows: The weighed quantity of cornstarch was placed in a pail and mixed with a little milk, then the bulk of the milk was added and the mixture well stirred. The calf consumed the mixture with eagerness and without any apparent digestive disturbance. To make sure that all the starch was consumed, the pail was rinsed once or twice with milk and the calf permitted to drink the rinsings. The starch-feeding period was followed by a rest period of about five days, during which only whole milk was fed; then the calves again received starch in addition to their whole-milk ration exactly as in their first 3-day period. The length of the periods and the duration of the

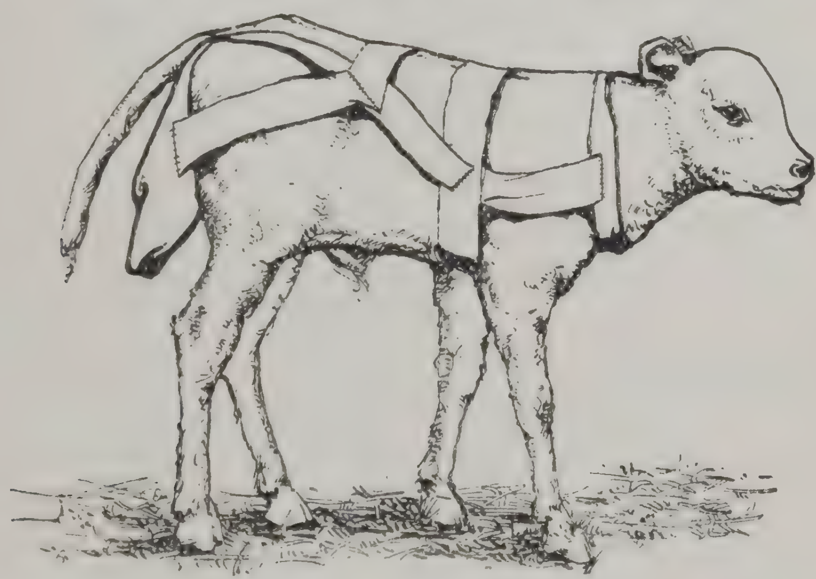

FIG. 1.—Bag for receiving feces and harness for supporting it.

experiment are shown in Table I. The cornstarch fed to calf 1 contained, according to analysis, 77.02 per cent of pure starch, and that fed calf 2, 76.32 per cent. The feces were received in a closely fitting rubber bag supported by a harness as shown in figure 1. Collections were made during the three starch-feeding days and the three days following. The feces were removed from the bag each day and immediately dried on the premises in an electric oven at about the temperature of boiling water. The dried feces, representing a starch-feeding period and the three subsequent days, were united and ground in a mill. The analyses were made according to the methods adopted by the Association of Official Agricultural Chemists, the malt-diastase method being selected for the starch determinations.

The feeding was conducted at the Bureau of Animal Industry's Experiment Farm, Beltsville, Md., and the analytical work was done at the Dairy Division laboratories in Washington. The results are given in Tables I and II.

TABLE I.—*Composition of feces of calves*

Animal and period.	Age of calf when feces were collected.	Weight of dry feces.	Nitrogen in feces.		Ether extract in feces.		Starch in feces.	
	Days.	*Gm.*	*Gm.*	*Per cent.*	*Gm.*	*Per cent.*	*Gm.*	*Per ct.*
CALF 1:								
1	4	288.98	14.07	4.87	6.36	2.20	144.2	49.91
2	12	270.28	13.74	5.08	11.95	4.42	101.5	37.56
3	20	244.87	14.20	5.80	13.75	5.62	67.8	27.70
4	30	179.40	11.66	6.50	7.36	4.10	15.2	8.49
5	39	202.00	17.25	8.54	14.71	7.28	2.2	1.07
CALF 2:								
1	4	283.49	14.27	5.03	12.08	4.26	146.0	51.48
2 [a]	14	....	....	....	....	....	....	....
3	23	190.21	13.54	7.12	24.25	12.75	6.7	3.53
4	31	152.95	10.28	6.72	17.58	11.49	1.6	1.03

[a] Sample was lost at the farm.

TABLE II.—*Proportion of starch digested by calves*

Animal and period.	Starch fed.	Starch in feces.	Starch digested.	
	Gm.	*Gm.*	*Gm.*	*Per cent.*
CALF 1:				
1	184.9	144.2	40.7	22.02
2	184.9	101.5	83.4	45.11
3	184.9	67.8	117.1	63.34
4	184.9	15.2	169.7	91.79
5	184.9	2.2	182.7	98.81
CALF 2:				
1	183.2	146.0	37.2	20.30
2 [a]	183.2	....	....	....
3	183.2	6.7	176.5	96.32
4	183.2	1.6	181.6	99.10

[a] Sample was lost at the farm.

CONCLUSIONS

The figures in Table II for digested starch show that the calves when from 4 to 7 days old were able to digest about one-fifth of the quantity consumed; in one case 22.02 per cent and in the other 20.30 per cent. When calf 1 was 12 to 15 days old, the percentage of starch digested had more than doubled and when 3 weeks old it had nearly tripled, while at 4 weeks in the case of calf 1 and at 3 weeks in calf 2, the percentage of starch digested was well over 90.

While it is quite probable that a calf but a few hours old can not digest an appreciable amount of starch, it can readily be seen that the quantity of starch-splitting enzyms must increase very rapidly in the first few days of life, for the calves under experiment, when only 3 to 4 weeks old, were able to digest a ration nearly 10 per cent of the dry matter of which was starch.

These results indicate that the milk ration of a calf but a few days old may be supplemented with a starchy food and that the starchy material may be rapidly increased as the calf grows older.

TOXICITY OF VOLATILE ORGANIC COMPOUNDS TO INSECT EGGS[1]

By WILLIAM MOORE, *Head of Section of Research in Economic Zoology*, and SAMUEL A. GRAHAM, *Assistant in Entomology, Minnesota Agricultural Experiment Station*

INTRODUCTION

A general survey of the literature has failed to reveal any extensive study of the toxicity of different materials to insect eggs. Certain spray solutions have been studied, but they have been considered individually and not in comparison with other related compounds.

Cooley (*1*)[2], working with the oyster-shell scale (*Lepidosaphes ulmi* L.) has shown that linseed oil, cottonseed oil, and lime-sulphur were effective. In the cases of the oils some of the eggs were killed, while other eggs hatched, but the young insects died during or shortly after emergence. Lime-sulphur failed to kill the eggs, but the young were killed very soon after hatching. Pure kerosene apparently had no effect on the eggs.

Gillette (*2*), working with aphid eggs of different species, has shown that eggs treated with kerosene emulsion containing less than 25 per cent of keresene were unaffected by the spray. Scalecide, Thompson's Soluble Oil, lime-sulphur, and different soaps had little effect unless used in very large doses. Tobacco extracts containing nicotine or nicotine sulphate were found to be very effective.

Safro (*9*), in the study of lime-sulphur as an ovicide for the codling moth (*Carpocaspa pomenella* L.), shows that this material is only effective to eggs in which the embryo is almost fully developed.

Woodworth (*10*) has studied the toxicity of hydrocyanic-acid gas to the eggs of scale insects, but does not consider the factor of age.

Recently particular attention has been given to the study of the toxicity of volatile organic compounds to the eggs of lice (*Pediculus capitis* and *P. corporis*, and *Phthiris pubis*). Kerosene has been used against head lice and their eggs for many years, but in the recent work many other materials have been recommended.

Postnikov (7) recommends amyl alcohol, ethyl alcohol, benzene, chloroform, carbon tetrachlorid, methane, and birch tar for the destruction of head lice and their eggs. Gasoline has been used for the destruction of the eggs of the clothes louse, and Kinloch (*4*) claims that immersion in this material for one minute will kill, while exposure to its vapor is fatal in one-half hour. He considers that benzene, toluene,

[1] Published, with the approval of the Director, as Paper 88 of the Journal Series of the Minnesota Agricultural Experiment Station.

[2] Reference is made by number (italic) to "Literature cited," p. 586–587.

and acetone are as toxic as gasoline. Von Prowazek (*8*) recommends xylol and ether for the destruction of lice and their eggs.

In view of the work of the senior author (*5, 6*) showing that the toxicity of organic compounds to insects is related to their volatility, of which the boiling point is a general index, it was thought advisable to make a similar study of the toxicity of a series of volatile organic compounds to insect eggs.

METHOD OF EXPERIMENTATION

For these experiments it was considered desirable to use eggs which were not protected from the action of the chemical by any covering. Eggs of the bedbug and the clothes louse were considered; but, owing to the fact that many of these eggs were found to be infertile under the artificial conditions of breeding, and, further, that they were hard to obtain in large enough quantities for the purpose of the experiments, they were discarded. Potato-beetle eggs (*Leptinotarsa decemlineata* Say) were finally decided upon as fulfilling all requirements. As many as 50,000 eggs were used in these experiments, and it was found that in every case untreated eggs hatched 100 per cent. They were also convenient to use, as a cluster of 20 to 30 or more eggs could be easily treated as a unit. The effects of the chemicals were studied in three different ways:

1. By dipping the clusters in the chemical to be tested.
2. By spraying the clusters with the chemical by means of an atomizer.
3. By exposing the eggs to the action of the vapor of the chemical.

In the exposure to the vapor the eggs were fumigated in a similar manner to that employed in the study of the toxicity of the vapor to houseflies recorded in a previous paper (*6*). It was found necessary, however, to use a longer time limit than 400 minutes, 15 hours being finally selected for this purpose.

Eggs were fumigated for 15 hours with varying quantities of the chemical, after which they were removed from the flask and placed in open pasteboard pill boxes until they hatched or were undoubtedly dead. The smallest dose necessary to kill the eggs in this length of time was thus determined and reduced to millionths of a gram-molecule, making possible an accurate comparison of the different chemicals used. In experiments where the eggs were dipped or sprayed they were placed in open pill boxes after treatment and handled in a manner similar to those fumigated.

RESULTS OF THE EXPERIMENTS

The results of dipping and spraying the eggs are given in Table I. The compounds used are arranged in the order of their boiling points, from the lowest to the highest. It will be noted that in general the eggs treated with compounds having the lowest boiling point—that is, the most volatile compounds, permitted most, if not all, of the eggs to hatch. Exceptions may be noted of compounds extremely active

chemically, such as allyl alcohol, which contained ammonia as an impurity, and chlorpicrin. In general those sprayed showed a higher percentage of hatching than those dipped. Some compounds of this series, the vapor of which had been previously shown (*6*) to be nontoxic to houseflies, owing to the fact that they formed gummy masses on exposure to the air, were found to be toxic to the insect eggs. Pinene, terpineol, and geranyl acetate are examples of such chemicals. Further, it is noted that compounds which are so slightly volatile, owing to their high boiling point, that they were ineffective against flies, were found to be toxic to the insect eggs. Such chemicals were eugenol, alpha naphthol, ethyl ether, and trimethylene cyanid. These compounds are only effective, however, when brought into actual contact with the eggs, as in spraying or dipping, and are no more effective as a fumigant against the eggs than they are against adult insects.

TABLE I.—*Relation of the boiling point to the toxicity of organic compounds used in dips and sprays for potato-beetle eggs*

Organic compound.	Boiling point.	Percentage hatching from eggs dipped and removed immediately.	Percentage hatching from eggs sprayed.	Organic compound.	Boiling point.	Percentage hatching from eggs dipped and removed immediately.	Percentage hatching from eggs sprayed.
	°C.				°C.		
Ethyl ether	35	100	100	Trimethylene bromid	165	0	....
Ethyl mercaptan	36.2	97	100	Terpineol	168	0	....
Carbon bisulphid	46	.....	100	Benzonitrile	170	0	....
Petroleum ether	40–70	100	....	Thiophenol	172.5	0	....
Acetone	56.3	88	100	Benzaldehyde	179.1	0	....
Chloroform	61	95	100	Anilin	182	0	....
Methyl alcohol	66.5	85	100	Ortho-bromtoluene	182	17	0
Carbon tetrachlorid	78.1	.....	100	Valeric acid	184.5	0	....
Ethyl alcohol	78.4	100	100	Ortho-creosol	190	0	....
Gasoline	70–90	44	88	Iodobenzene	193	0	....
Benzene	80.3	100	100	Salicylic aldehyde	196	0	....
Thiophene	84	100	0	Para-cresol	201.8	0	....
Allyl alcohol	97	0	....	Meta-cresol	202.8	0	....
Amyl nitrite	98	50	0	Nitrobenzene	205	0	0
Nitromethane	101	100	....	Benzyl alcohol	206.5	0	....
Propyl acetate	102	100	100	Kerosene	150–300	83	100
Toluene	111	0	100	Ortho-nitrotuluene	223	0	....
Chlorpicrin	112	0	0	Bromxylene	225	.....	0
Pyridin	116.7	.....	16	Citral	225	0	....
Acetic acid	119	100	0	Quinolin	239	0	....
Chlorbenzene	132	0	....	Eugenol	247.5	0	....
Amyl alcohol	137	0	20	Nitroxylene	250	0	....
Xylene	140	74	100	Nicotine	250	0	0
Amyl acetate	148	.....	100	α-Napthol ethyl ether	272	0	....
Bromoform	151.2	.....	20	Trimethylene cyanid	274	0	....
Pinene	160	0	....	Geranyl acetate	....	0	....
Ethyl malonate	160	0	0	Brommethylphenylketone	....	0	....
Allyl isosulphocyanate	161	.....	0	Ethyl aceto-acetate	....	0	....
Furfural	162	0	0				
Butyric acid	163	0	0				

Kerosene, although having a high boiling point of 150° to 300° C., allowed 83 per cent of the dipped eggs and 100 per cent of the sprayed eggs to hatch.

TABLE II.—*Toxicity of various organic compounds to eggs of different ages when dipped for various periods*

Compound.	Age.	Percentage hatching after dipping for—						
		1 second.	5 second.	10 seconds.	15 seconds.	30 seconds.	60 seconds.	120 seconds.
Ether	Fully developed embryos	100	100	100	100	....	....	....
	Partially developed	100	0	100	100	....	....	....
	Freshly laid	0	0	0	0	....	....	....
Carbon bisulphid	Fully developed	100	100	0	100	....	....	....
	Partially developed	100	0	0	0	....	....	....
	Freshly laid	100	0	100	0	....	....	....
Methyl alcohol	Fully developed	100	100	100	....	....	100	100
	Partially developed	100	100	100	....	....	100	100
	Freshly laid	100	100	100	....	....	0	100
Chloroform	Fully developed	0	100	100	100	0	100	0
	Partially developed	100	100	100	100	0	0	100
	Freshly laid	....	100	0	0	100	0	0
Carbon tetrachlorid	Fully developed	....	3	....	12	0	5	0
	Partially developed	....	100	....	100	100	100	100
	Freshly laid	100	0	....	0	100	100	0
Benzene	Fully developed	100	100	....	....	100	....	....
	Partially developed	100	0	....	....	0	....	....
	Freshly laid	100	0	....	....	100	....	....
Toluene	Fully developed	100	100	....	....	0	....	....
	Partially developed	100	100	....	....	100	....	....
	Freshly laid	100	100	....	....	100	....	....
Acetic Acid	Fully developed	0	0	....	....	0	....	....
	Partially developed	0	0	....	....	0	....	....
	Freshly laid	0	0	....	....	0	....	....
Xylene	Fully developed	100	0	....	....	0	....	....
	Partially developed	100	100	....	....	32	....	....
	Freshly laid	100	100	....	....	0	....	....
Ortho-bromtoluene	Fully developed	0	0	....	....	0	....	....
	Partially developed	0	0	....	....	0	....	....
	Freshly laid	0	0	....	....	0	....	....

In the experiments it was noted that compounds with low boiling points evaporated from the surface of the eggs very quickly, this giving the material very little time to penetrate. On the other hand, compounds with high boiling points remained upon the eggs for hours, or even days. In view of this fact, a series of experiments was conducted with the lower boiling-point compounds, in which the eggs were dipped

for periods from 1 to 120 seconds, eggs of different ages being used. The results are given in Table II. This table shows that in general with compounds of very low boiling points the freshly laid eggs or those containing embryos only partially developed were more easily killed than those in which the embryo was fully developed. Gortner and Banta (*3*), in working with the toxicity of certain phenolic compounds to amphibian eggs, found that the youngest eggs were more susceptible than the older eggs. This may be due to disturbances in the permeability of the egg, and compounds with low boiling points such as ether would have more influence on the permeability than compounds with higher boiling points. With an increase in the boiling point it was found that the eggs in which the embryo was fully developed were most easily killed. Compounds with very high boiling points, such as nicotine and kerosene, often remained on the eggs and killed the larvæ in the act of hatching. It seems that compounds with high boiling points are not able to penetrate the egg as readily as compounds having low boiling points. In general a slightly longer exposure to the chemical will result in the death of a larger number of eggs.

In these experiments the writers they have been unable to remove compounds of high boiling points from the surface of the egg without injury to the egg, and, hence, have no data as to the length of time necessary to kill with these compounds.

TABLE III.—*Toxicity of kerosene to eggs of various ages*

Age of eggs.	Percentage hatching after dipping for—					
	1 min.	5 min.	10 min.	15 min.	30 min.	Brand.
1 day	0	0	100	0	......	Unknown.
2 days	......	100	100	100	......	Do.
3 days	100	0	100	100	......	Do.
4 days	......	13	100	100	......	Do.
Freshly laid	100	100	100	100	100	Do.
Slightly developed	100	100	0	0	100	Do.
Well developed	33	0	100	0	0	Do.
Spiracles visible through shell	0	0	0	0	0	Do.
Within 1 day of hatching	100	......	......	......	......	Pearl oil.
Within 2 days of hatching	100	......	......	......	......	Do.
Within 3 days of hatching	100	......	......	......	......	Do.
Within 4 days of hatching	100	......	......	......	......	Do.

A special study was made of the action of kerosene on the eggs, inasmuch as it was a high-boiling-point compound which did not always kill. The results of dipping eggs of different ages in kerosene are given in Table III. Eggs 1 day old in general did not hatch after being dipped. Eggs classed as freshly laid, which may have been 1 or 2 days old, hatched in every case, while eggs with well developed or fully developed embryos failed to hatch. This is interesting in that by killing the young eggs it

acts in a similar manner to compounds with a low boiling point, while by killing the eggs with fully developed embryos it acts as a compound having a high boiling point. The Pearl oil used in the experiments is a product from the California oil fields and therefore differs chemically from the other kerosenes used.

In order to obtain further data on the toxicity of kerosene to eggs, two brands of kerosene were broken up into fractions of different boiling points. The two brands used were Pennsylvania Best sold by the Pure Oil Co. and Perfection oil put out by the Standard Oil Co. of Indiana. The data obtained by this experiment are shown in Table IV. The fraction of Pennsylvania Best boiling between 140° and 187° C. killed only freshly laid eggs. The fraction between 187° and 234° C. killed both the fully developed and freshly laid eggs, while the fraction between 234° and 280° C. killed all the eggs. Apparently the lower fraction did not remain long enough upon the fully developed eggs to produce any influence. It was noted in the kerosene experiments that the higher fractions killed at the time of hatching. This may explain the fact that the partially developed eggs treated with the second fraction allowed 100 per cent to hatch. These eggs being too far developed were not effected by changes in permeability such as would kill a freshly laid egg, while the compound had sufficient time to evaporate from the surface of the egg before the emergence of the larva.

TABLE IV.—*Toxicity of different fractions of kerosene on eggs of different ages*

Brand.	Age.	Percentage hatching after dipping for 5 seconds in fractions with boiling points of—				
		140°–187° C.	187°–234° C.	234°–280° C.	Residue above 280° C.	Undistilled.
Pennsylvania Best..	Fully developed....	100	0	0	....	0
	Partially developed	100	100	0	....	0
	Freshly laid........	0	0	0	....	0
	General............	100	100	0	0	100
	.....do............	100	100	100	0	50

Brand.	Age.	Percentage hatching after dipping for 5 seconds in fractions with boiling points of—		
		135°–183° C.	183°–231° C.	231°–280° C.
Perfection	Fully developed..............	0	0	0
	Partially developed...........	100	100	0
	Freshly laid..................	0	0	0

The Perfection oil killed both the fully developed and freshly laid eggs, leaving the partially developed uninjured except for the fraction between 231° and 280° C. Other experiments with these two oils, which will be published later, have shown that the Perfection oil is more toxic than the Pennsylvania Best. Kerosene therefore has oils of low boiling points which kill the freshly developed eggs and oils of high boiling points which destroy the fully developed eggs, while the partially developed eggs are most likely to hatch.

Nineteen compounds were tested to determine the toxicity of their vapor to insect eggs. The results of these experiments are given in Table V, in which the compounds are arranged according to their boiling points, from the lowest to the highest. The increase in toxicity with an increase in boiling point is indicated by the smaller portion of a gram-molecule necessary to kill in 15 hours. That the boiling point is not so good an index of the toxicity of a chemical as its volatility is shown in Table VI, where the compounds are arranged according to their volatility, from the most volatile to the least volatile. A comparison of the two tables shows a better correlation between volatility and toxicity than between boiling point and toxicity. As was found in the work with the housefly (5), chemicals having low boiling points are more valuable for fumigation purposes due to the fact that a larger amount of the vapor may be contained in the air.

TABLE V.—*Toxicity of the vapor of certain organic compounds (in millionths of a gram-molecule required to kill in 15 hours' exposure). Compounds arranged according to their boiling points*

Compound.	Boiling point.	Toxicity (millionths of a gram-molecule).	Compound.	Boiling point.	Toxicity (millionths of a gram-molecule).
	°C.			°C.	
Ethyl ether	35	3,468.2	Toluene	111	104.0
Carbon bisulphid	46	555.5	Chlorpicrin	112	2.8
Petroleum ether	40–70	243.2	Chlorbenzene	132	160.8
Acetone	56.3	1,363.0	Xylene	140	29.5
Chloroform	61	558.0	Bromoform	151.2	3.9
Methyl alcohol	66.5	1,232.0	Furfural	162	1.8
Carbon tetrachlorid	78.1	404.0	Nitrobenzene	205	2.7
Ethyl alcohol	78.4	284.0	Nitroxylene	250	3.3
Gasoline	70–90	41.1	Nicotine	250	1.8
Thiophene	84	344.0			

TABLE VI.—*Toxicity of the vapor of certain organic compounds (in millionths of a gram-molecule required to kill in 15 hours' exposure). Compounds arranged from the most volatile to the least*

Compound.	Toxicity (millionths of a gram-molecule).	Compound.	Toxicity (millionths of a gram-molecule).
Ethyl ether	3,468.2	Chlorbenzene	160.8
Petroleum ether	243.2	Toluene	104.0
Methyl alcohol	1,232.0	Xylene	29.5
Acetone	1,363.0	Gasoline	41.1
Carbon bisulphid	555.0	Bromoform	3.9
Chloroform	558.0	Furfural	1.8
Carbon tetrachlorid	404.0	Nitrobenzene	2.7
Ethyl alcohol	284.0	Nicotine	1.8
Chlorpicrin	2.8	Nitroxylene	3.3
Thiophene	344.0		

Fumigation in a saturated atmosphere with ether, ethyl mercaptan, carbon bisulphid, benzene, carbon tetrachlorid, and chloroform will kill all the eggs in one hour.

SUMMARY

(1) In general, compounds with high boiling point and slight volatility are more effective in dipping and spraying insect eggs than compounds with low boiling point and high volatility.

(2) Compounds with low boiling points kill freshly laid eggs more readily than eggs in which the embryo is partially or fully developed.

(3) Compounds of higher boiling points are more toxic to eggs with fully developed embryos than they are to eggs in which the embryo is only slightly formed.

(4) Kerosene containing both high and low boiling points is destructive to both young and old, but is only slightly toxic to partially developed eggs.

(5) The toxicity of the vapor of organic compounds to insect eggs is related to the boiling point and the volatility. As the boiling point increases and the volatility decreases, the toxicity increases.

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CORN-STOVER SILAGE

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INTRODUCTION

The ensiling of corn stover and cured corn fodder is not a new idea. In a few localities farmers have followed this practice to a limited extent for some years, and there have appeared several Experiment Station publications which deal briefly with the process. Experimental data on the subject are, however, very meager; little has been established with reference to the practicability of stover silage, while nothing is known concerning the nature of the fermentation which takes place and the factors operative other than what may be deduced from knowledge of ordinary silage. The present necessity for more economic production, with the conservation of concentrates and the utilization of more roughage in the live-stock and dairy industries, makes a reconsideration of corn-stover silage of especial pertinence.

The object of the present study was partly to test the practicability of ensiling stover, and partly to determine the nature of the fermentation which takes place therein.

PRACTICABILITY OF ENSILING CORN STOVER

The stover used in this experiment was ensiled early in April, 1916; the material had been kept in a shed since fall and was quite dry. The condition of the material was not good; it was moldy in spots and on the whole represented an inferior grade of stover. This stover was run through a silage cutter and packed in the silo by means of tramping. Water was added in a continuous stream through a hose which was carried and the water distributed by the man who did the packing. A water meter was attached to the hose so as to enable the regulation of the amount used. A wooden-stave silo 16 feet in diameter was filled with 32,000 pounds of stover to which were added 66,000 pounds of water.

Samples of the silage, which were taken at frequent intervals, were examined for general appearance, texture, and aroma. The stover was soon observed to undergo a fermentation with the formation of a product quite similar to normal silage made with green corn. The material softened, regained a slightly greenish color, and developed an aroma simulating that of normal silage, though inferior in all these respects to silage made in the usual way from green corn.

Feeding tests made at the end of the experiment showed that cattle ate this silage with little waste and apparently with a relish. While

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it is not believed that the stover silage is as palatable as is that made from fresh corn, it did prove to be a very acceptable feed which was preferred by cattle to any of the dry roughages furnished. Some experiments in which the actual feeding value of stover silage is determined in comparison with ordinary silage and other roughages are desired, and it is hoped that such tests may be carried out at this Station in the near future.

The keeping quality of stover silage appears to be excellent, provided sufficient water is added. Although, as is the case with other types of silage, the surface material undergoes a moldy spoilage accompanied with heat formation, this condition does not extend to more than the ordinary depth. The silage made in this experiment was not all used during the following winter, and the remaining material, at the time of this writing nearly 1½ years old, is still in excellent condition. In view of the very satisfactory results obtained with such an inferior raw product, we do not hesitate to predict success in the ensiling of any stover which is in reasonably good condition.

Probably the most important consideration for the successful production of stover silage is the amount of water to add. This obviously will vary according to the quantity of water contained in the stover, and this factor should be taken into consideration. While it would be more scientific and exact to determine the most desirable amount of water to add by means of moisture tests on the stover, such a recommendation would find no place in farm practice. In our experimental silo the proportion was about 2 parts of water to 1 of stover, but the stover was probably somewhat drier than would usually be the case. As may be seen from the moisture determinations, which are reported in another section (Table I), the quantity of water added was none too much; in general appearance and to the touch some of the samples seemed to be considerably below the most desirable point.

A laboratory test was carried out upon this point by making stover silage in small jars with varying quantities of water and examining after about one month for general appearance and condition of moisture. The stover used in these tests was very dry. Samples which were made with equal parts by weight of water and stover, as well as those made with 1½ parts of water to 1 of stover were too dry to undergo a typical fermentation and form good silage. Those which had water in the proportion of 2 parts to 1 of stover made good silage, but did not appear to have as much moisture as would be best. Samples put up with 2½ and with 2¾ parts by weight of water to 1 part of stover were in good condition when opened and apparently did not contain an excess of water.

It seems that in general, when reasonably fresh stover is used, about 2 parts of water by weight to 1 of stover would be advisable, while for older and drier stover a slightly larger proportion of water may be more

desirable. It should be kept in mind that these tests were all made with rather dry stover. In the case of ensiling soon after the corn is husked, 2 parts of water might be too much. However, it appears from our observations that there is less danger of adding too much water than of getting too small an amount, and that considerable water may be added above the required amount without injury to the product.

The water should be added uniformly as the silo is being filled so that all the dry cut stover becomes thoroughly wet down. If this precaution is not taken, the water may follow channels down through the silage and waste away at the bottom of the silo. In such an instance spoiled silage might result in some parts of the silo because of an insufficient amount of water.

FERMENTATION OF STOVER SILAGE

To obtain information on the nature of the fermentation which takes place in silage made from stover, determinations were made of the volatile and nonvolatile acids, temperatures, and numbers and types of bacteria at various stages of the ripening process.

Samples for examination were obtained by means of a 2-inch auger provided with an extension shaft of 8 feet, thus making it possible to penetrate to the center of the silo. By repeatedly boring in a short distance and withdrawing the auger until the center of the silo was reached, no difficulty was experienced in securing sufficient material for the different tests. The sample obtained in this way represented the silage mass from the wall to the center of the silo. Not more than one boring was made in one place, the different samples being removed at points all the way around the silo and from 3 to 8 feet from the ground. The material so obtained was subjected to pressure in an ordinary lard press and sufficient juice collected for the various examinations made.

Acid formation.—There can be no doubt that the amount and character of acids in silage influence its quality profoundly. Since it has been the general experience that extremely green corn produces a very sour silage, while corn more nearly mature produces a silage with less acid and of a much better quality, the acid fermentation in silage made from dry material is of interest.

The volatile acidity was determined by subjecting a 100-gm. sample of juice to steam distillation under reduced pressure until 4 liters of distillate were secured. These were titrated directly after collection with $N/10$ barium hydrate, with phenolphthalein as the indicator. The nonvolatile acidity was obtained by the difference between the volatile acidity and a total acid determination made by the titration of 20 gm. of juice, diluted to 500 c. c. with carbon-dioxid-free water, against $N/10$ barium hydroxid. In Table I the nonvolatile and volatile acids (calculated as lactic and acetic, respectively) are reported in terms of percentage of air-dry material.

TABLE I.—*Acid formation in stover silage*

Age.	Total solids.[a]	Acid in total solids.		Ratio of nonvolatile to volatile.
		Nonvolatile.[b]	Volatile.[c]	
Weeks.	*Per cent.*	*Per cent.*	*Per cent.*	
3/7	28.9	Trace	0.51	....
1	35.5	0.16	.87	1 : 5.40
2	26.7	.95	1.36	1 : 1.43
3	26.8	1.36	1.41	1 : 1.04
4	27.1	1.54	1.49	1 : .97
5	29.7	1.51	1.55	1 : 1.02
6	31.2	2.00	1.69	1 : .85
8	27.5	2.53	1.92	1 : .75
10	32.1	2.64	1.82	1 : .75
12	25.8	3.15	2.24	1 : .71

[a] Air-dried. [b] Calculated as lactic acid. [c] Calculated as acetic acid.

It is seen from the figures in Table I that for the first week the volatile acidity was greatly in excess if the nonvolatile portion; that from the second to the fifth week the two were apparently present in about equal amounts; and that from the sixth week on, the nonvolatile acids were in excess, the proportion of nonvolatile acidity increasing to the end of the experiment. The total acidity obtained was somewhat lower than is usually found in ordinary silage. This is probably to be expected, in view of the chemical differences in the raw materials. In this connection, however, it should be noted that in this experiment samples were not taken after the twelfth week. It is not unlikely that the acidity increased some after the last sample was secured. In regard to the proportion of nonvolatile to volatile acids, if we accept the ratio 1 to 0.75 reported by Dox and Neidig (*5*)[1] as representing a general average for ordinary silage, it will be seen that our results on corn stover silage indicate a remarkable agreement in this respect between these two types of ensilage.

FERMENTATION TEMPERATURES.—Temperature records were obtained by means of four resistance bulbs with about 60 feet of insulated cable attached to each which were buried in the silage as the silo was filled. The bulbs were located as follows:

No. 1. About 2 feet from the bottom and in the center of the silo.

No. 2. About 6 feet from the bottom and about 3 feet from the center of the silo.

No. 3. At the same height as No. 2 but about 3 feet from the center in the opposite direction.

No. 4. About 12 feet from the bottom and in the center of the silo.

The ends of the cables were located at a convenient place on the outside of the silo so as to allow easy attachment for temperature readings. Table II gives the temperatures obtained from April 4, the day the silo was filled, until June 16.

[1] Reference is made by number (italic) to "Literature cited," p. 600.

TABLE II.—*Fermentation temperatures of stover silage*

Date.	Temperature (°F.).				
	Bulb 1.	Bulb 2.	Bulb 3.	Bulb 4.	Atmosphere.
Apr. 4	42.0	42.0	42.0	....	33.5
5	42.5	49.5	47.0	....	42.0
6	46.5	50.5	48.0	46.5	43.0
7	46.5	51.0	48.5	47.5	46.5
8	47.0	52.5	49.5	48.0	33.0
9	47.5	54.0	51.0	49.5	33.0
10	49.0	54.0	51.0	50.5	37.0
11	49.0	54.5	51.5	51.0	42.0
12	49.5	54.5	52.0	52.5	48.0
13	50.0	56.0	52.5	52.5	58.0
14	50.5	56.0	53.5	55.0	51.0
15	50.0	56.0	53.5	55.0	49.0
16	50.0	56.0	53.5	55.0	50.0
17	50.0	56.0	53.5	55.5	52.0
18	50.0	56.5	54.0	56.0	49.5
19	50.0	57.0	54.5	56.0	46.0
20	50.0	57.0	54.5	56.0	55.0
21	50.0	57.0	54.5	55.0	49.5
22	49.5	57.0	54.5	56.0	49.5
23	49.5	57.5	55.0	57.0	41.5
24	50.0	57.5	55.0	57.0	47.0
25	50.0	57.5	55.0	57.0	47.0
26	49.5	57.5	55.0	57.5	48.5
27	49.5	57.5	55.0	57.5	48.5
28	50.0	57.5	55.5	57.5	47.0
May 1	49.5	57.0	55.5	58.5	66.5
2	49.0	57.5	55.5	59.5	63.5
4	50.0	58.5	56.5	59.5	53.5
6	50.0	58.5	56.5	59.5	60.5
8	50.0	58.0	56.0	59.0	65.5
16	50.5	58.0	56.0	59.0	65.0
22	50.5	58.0	56.5	59.0	55.0
31	51.0	58.0	57.0	61.0	64.0
June 16	52.0	58.5	57.5	63.0	63.5

Recent investigations have furnished abundant proof that high temperatures are not essential in silage preservation, and, in fact, do not occur except at the surface, which undergoes an aerobic decomposition. Bechdel (*3*) has recorded an instance in which the maximum temperature attained in the center of a concrete silo during the curing period was only 60° F. As has been shown by Eckles, Oshel, and Magruder (*6*), the atmospheric temperature at time of filling influences greatly the temperature attained during the fermentation of the silage.

Table II shows that the temperature at the start was 42° F. and gradually increased until the readings were discontinued. The maximum temperature attained was 63° F. in the case of bulb 4; but bulb 1, which was buried to a depth slightly below the surface of the surrounding soil, showed a maximum temperature of only 52° F. An examination of the column giving the atmospheric temperature during this period suggests that the continued increase in the silage temperature during the latter part of the time may be accounted for by a similar increase in

the outside air. However, the more rapid increase during the early part of the period was entirely independent of this factor. A comparison of the temperature records secured in this experiment with the data which have been obtained on ordinary silage at this and other Experiment Stations indicates that there is no wide difference, if any, between the rate and amount of increase in temperature in silos in which ordinary silage and corn-stover silage have been stored.

BACTERIOLOGICAL OBSERVATIONS.—A quantitative bacterial examination was made on each of the samples taken. The juice obtained was plated in proper dilutions and the counts obtained were reported as numbers per cubic centimeter of juice. Lactose agar was used, and the plates were counted after six days' incubation at 33° C. As may be seen from Table III, it appears that the bacterial count increases during the first week and is followed by a continued decrease thereafter.

TABLE III.—*Number of bacteria in stover silage at different stages of curing*

Age.	Number of bacteria per cubic centimeter.	Age.	Number of bacteria per cubic centimeter.
Weeks.		*Weeks.*	
3/7	528, 000, 000	5	510, 000, 000
1	3, 630, 000, 000	6	235, 000, 000
2	1, 850, 000, 000	8	186, 000, 000
3	975, 000, 000	10	98, 000, 000
4	400, 000, 000	12	71, 000, 000

Direct microscopic examinations of the silage juice were made in order to follow in a general way any marked changes which take place in the bacterial flora during the curing process. This at best could only give suggestive data, but such examinations are sometimes important in connection with cultural studies. At first a great variety of cells were observed. During the first two weeks rods and cocci were apparently present in about equal numbers, after which the rods became increasingly predominant. Toward the end of the experiment practically nothing but rods were found in the microscopic preparations. Because of the high acidity it is not likely that the cocci were active nearly as long as they appeared under the microscope. But it is probable that the acid medium would tend to peserve the cells so that they would appear for some time after they were inactive or even dead.

A qualitative bacterial study was also carried out. All of the colonies from a representative lactose-agar plate from each sample were isolated and subjected to a cultural study. For the present purpose they may be divided roughly, according to their action on litmus milk, into acid-forming, casein-digesting, alkali-forming, and inert groups. The acid formers may be further divided according to whether they produced sufficient acid to cause coagulation of the milk. The distribution of these groups in silage at various stages of its fermentation is shown in Table IV.

TABLE IV.—*Groups of bacteria present at different stages of curing*

Age.	Percentage of total number.				
	Acid-coagulating group.	Acid-noncoagulating group.	Casein-digesting group.	Alkali-forming group.	Inert group.
Weeks.					
3/7	10	53	10	4	23
1	14	57	9	6	14
2	20	71	0	0	9
3	11	63	5	0	21
5	21	67	0	0	12
6	60	30	0	0	10
8	68	32	0	0	0
10	60	40	0	0	0
12	56	39	5	0	0

Table IV shows that the rather complex bacterial flora which is present at the beginning of the process gives way to one which is almost entirely acid-producing as the fermentation progresses. The proportion of acid-forming and coagulating organisms to the noncoagulating ones also increases as the curing period advances. A comparison of these figures with those given in Table III indicates that the change in flora is not to be accounted for by an actual increase in the high acid-forming organisms during the latter part of the fermentation period, but rather to the fact that they do not decrease as rapidly because of their greater resistance to the unfavorable hydrogen-ion concentration.

The division of the acid-forming organisms into coagulating and noncoagulating types, though convenient and significant for the present purpose, probably does not separate them into natural groups. From early in the fermentation the predominating organisms were acid formers, most of which probably belonged to the same general group. We have found cultures which were apparently identical, as indicated by the cultural and fermentative reactions studied, but which varied in the amount of lactic acid produced in milk from only 0.3 to more than 2.0 per cent. All of these probably belonged to the same general group as the aciduric bacteria which have previously been noted as occurring abundantly in silage. In the first two samples examined organisms of the colon-aerogenes group were found and also a few cultures which were probably *Streptococcus lacticus*, but tests were not applied which would definitely identify the latter.

NATURE OF SILAGE FERMENTATION

Until recently the cell-respiration theory of silage fermentation established by Babcock and Russell (*1*, *2*) has not been seriously challenged. During the past year, however, several publications have appeared in support of the bacterial explanation of this phenomenon. In view of the recent contributions to the subject, it is not out of place to examine

critically the present status of the question. As the older literature has been reviewed so many times that further elaboration is not necessary, we shall pass in review only those papers of very recent date.

Hunter and Bushnell (*8*) have demonstrated the presence in silage of large numbers of high acid-producing bacteria, and have furnished strong evidence that these organisms are mainly responsible for the acid fermentation. Although their work is a most valuable one, it should be borne in mind that the evidence is circumstantial and perhaps not conclusive. It is rather doubtful if the data submitted justify the positive conclusion that—

> The present investigation warrants the statement that acid production, common to all normal silage, is largely the result of fermentation by the Bulgarian group of bacteria.

The fact that these bacteria formed considerable acetic acid when grown in alfalfa extract to which was added 1 per cent of glucose hardly warrants the assumption that—

> Although these organisms evidently do not produce all of the acetic acid found in normal silage, they must be responsible for a large per cent of it.

Sherman (*13*) also noted the presence of large numbers of the aciduric bacilli in silage and reported some observations which indicated that they are of significance in the fermentation process. The evidence, however, was not direct and by no means conclusive.

Hunter (7) in his work on heat production in silage has added further weight to the bacterial theory of silage fermentation. Unfortunately it is not possible to evaluate properly some of the interesting points contained in his paper, as they are obscured by insufficient description. For example, some data are given which show the difference in heat formation between green kafir heated and green kafir inoculated with *Bacillus bulgaricus*. The exact treatment in this case is not clear: If the inoculation was made into heated kafir, the results are of utmost significance; if, on the other hand, as the caption of the graph would indicate (*see* 7, *fig.* 9), the inoculation was made into unheated kafir and that compared with heated kafir, the test contributes nothing to the solution of the moot question. In his assumption that cell respiration can play no part in the fermentation of silage made from dry forage, Hunter has arrived, we think, at conclusions which are, in part at least, erroneous.

In a very interesting paper Lamb (*9*) concludes that both factors are of importance, but that microorganisms play the larger part, especially in the production of acid. Following the suggestion of Rahn (*10*), he has attempted to determine the cause of the process by the rates of change in the fermentations studied. The course of the curve obtained when such data were plotted was interpreted as indicating whether the action was of bacterial or enzymic origin.

Although it is true that purified enzym preparations acting under favorable conditions give time curves which follow, with certain modifications, the law of mass action, while curves representing bacterial action take an entirely different course because of an increase in the active mass with the multiplication of the organisms, we are inclined to believe that Lamb has placed undue confidence in this method, especially when we consider the complexity of the material studied and the factors concerned. A few considerations will suffice to illustrate some of the possibilities of error in such a method. In the first place conditions in silage are not constant, but are undergoing continual change. For example, the temperature and acidity, which are of utmost significance in enzym action, increase as the fermentation progresses. In view of the great increase in activity of some enzyms as the temperature is increased, and the stimulating effect on some enzyms of an increased hydrogen-ion concentration (within certain limits), it is not at all impossible that these factors might so modify the course of action of an enzym as to produce a curve resembling that typical of bacterial action.

Again, the phenomenon of adsorption and the action of the so-called antienzyms in many cases may so suppress the activities of an enzym during the early stages of the reaction as to cause it to follow a course not at all characteristic of enzym action. This has been beautifully illustrated by Rosenthal (*11*) in his work on the antitryptic action of egg albumen. It was shown that the trypsin was at first suppressed, but gradually regained its power and increased in activity so as to give the appearance of an increase in the "active mass" as indicated by a curve convex to the axis of abscissæ (the typical bacterial curve). On the other hand, the same trypsin preparation when acting on egg albumen which had been previously heated to destroy the antitrypsin gave a time curve characteristic of enzym action.

These illustrations will suffice to show the fallacy of such a method, but the number of possibilities of error in its application to such a complex mixture as silage might be increased almost indefinitely. That the limitations of this method of studying biochemical phenomena were appreciated by Rahn (*10*) is shown in the following paragraph from his valuable paper:

> It is hardly necessary to mention that the curve of a process will be an absolute means of discussion only in case of pure cultures. In natural fermentations, there is always the possibility that different processes taking place at the same time destroy the regular form of the curve. A simple example would be the growth of an acid-producing and an alkali-producing organism in the same liquid. It is also possible that an enzymic curve under certain conditions shows the form of a fermentation curve. We can imagine that an enzym is acting slowly at first, because of an unsatisfactory acidity of the medium. By a chemical or microbial process, independent of the enzymic action, the acidity may be made more suitable for the enzyme, and this will cause an increased rate of action of the enzyme and give the type

of a fermentation curve without the presence of organisms. The value of the curve is, therefore, not an absolute one and no conclusions ought to be drawn without consideration of the possibilities of error.

It would indeed seem that the application of such methods to the study of silage fermentation is entirely without foundation.

To the evidence in support of the bacterial theory of silage fermentation should be added the very suggestive observation of Clark (*4*) that the hydrogen-ion concentration of mature silage is coincident with the limiting hydrogen-ion concentration obtained in cultures of *Bacillus bulgaricus*, which organisms are considered by some workers as of paramount importance in the ripening process. In support of the respiration theory, on the other hand, it is pertinent to call attention to the recent work of Round (*12*), which indicates that cell respiration is of greater importance in the fermentation of sauerkraut than has been generally recognized.

It was thought at the beginning of this experiment that a study of the fermentation in silage made from dry stover would throw much light on the nature of the process in ordinary silage. The belief was held that the activity of the plant cells (which have been demonstrated to play an important part in the fermentation of silage made from green corn) would be eliminated, to a large extent at least, in the stover silage. But, as may be seen from an examination of the foregoing results, the fermentation of ensiled stover appears to resemble, in its main characteristics, that which takes place in green-corn silage. In an effort to determine the factors responsible for the fermentation, laboratory tests were made by ensiling stover under different conditions. Glass jars containing 175 gm. of cut stover and 400 gm. of water were used. Some were untreated, some put up with antiseptics, while some were sterilized in the autoclave and reinoculated with 1 per cent of raw-silage juice. The results of this study are given in Table V.

TABLE V.—*Fermentation of stover ensiled under different conditions*

Sample No.	Treatment.	Age.	Acidity.	Bacterial count.
		Weeks.	*Per cent.*	
1	Untreated	4	15.5	210,000,000
2	....do	4	10.4	240,000,000
3	Sterilized and inoculated	7	2.4	320,000,000
4	....do	5	3.3	36,000,000
5	2 per cent of toluene	6	4.3	180,000
6	....do	6	4.6	460,000
7	2 per cent of ether	5	10.8	7,500,000
8	....do	8	12.4	Not made.

The main points brought out by this test are that stover silage is capable of undergoing a fermentation when preserved with ether, while bacteria alone are apparently unable to produce the typical fermentation, even though conditions are favorable for their active development. The predominating organisms in the sterilized and inoculated samples

were of the same type as those characteristic of normal silage. The organisms found in the samples preserved with antiseptics, on the other hand, were a more miscellaneous group; and, although many probably belonged to the same group as the aciduric bacilli of normal silage, the cultures isolated were mostly very weak acid producers. The fact that fermentation took place under ether indicates that the activity of the plant cells, whether it be called "respiration" or "autolysis," is present in silage made from dry stover. As silage preserved with ether fermented, whereas in that kept with toluene the process was checked suggests that conclusions drawn from experiments conducted with only one antiseptic are of doubtful value. When opened, the ether-preserved samples, after the evaporation of the ether, appeared to resemble the untreated material, while the sterilized and inoculated silage were "flat" and lacking in the characteristic aroma. The results with ether were checked by another test in which triplicate samples were preserved, and again an active fermentation took place as was indicated by the development of acidity in each case.

Although our results would tend to support the respiration theory of silage-curing rather than the bacterial, we do not feel that the data thus far collected warrant definite conclusions on this point. It is difficult to believe that such active acid-forming organisms should occur in silage in large numbers without taking some part in the acid fermentation; perhaps they supplement in some important way the action of the plant cells. It is not inconceivable that a preliminary cleavage due to cell respiration is an essential prerequisite for the vigorous action of the aciduric bacteria. In fact, the continued increase in the ratio of nonvolatile to volatile acidity as the fermentation progressed (see Table I) might lead one to suspect that such was the case. On the other hand, the great increase in the nonvolatile acidity from the fifth to the twelfth week, during which time the bacterial count was rapidly decreasing, might be interpreted as strong evidence against that view. It is clear that microorganisms are not solely responsible for the fermentation of silage, and the weight of evidence at the present time, in our opinion, indicates that their rôle is not as important as that of the plant cells.

Although not committing ourselves definitely on the nature of silage fermentation in general, in regard to the present problem we do maintain that the fermentation which takes place in stover silage is similar in its essential points to that of ordinary silage and is caused by similar factors.

SUMMARY

Corn stover when ensiled with a suitable quantity of water undergoes fermentation with the production of a palatable silage of good keeping quality, which resembles ordinary corn silage in aroma and appearance.

The fermentation which takes place in corn-stover silage appears to be essentially the same as that of silage made from green corn, as is

indicated by the total acidity developed, the ratio of nonvolatile to volatile acids, temperature observations, and bacterial studies.

From a review of the present status of the question as to whether bacteria or plant cells are mainly responsible for silage fermentation, it is concluded that the data thus far published are inconclusive. Although the results of the present study tend to support the cell-respiration theory, conclusions on this point are withheld.

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WEEVILS WHICH AFFECT IRISH POTATO, SWEET POTATO, AND YAM

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INTRODUCTION

In a previous article[1] the writer discussed three important Andean weevil pests of the potato tuber (*Solanum tuberosum*). In the present paper a fourth potato tuber weevil is described and notes are presented on three weevils which attack the tubers of sweet potato (*Ipomoea batatas*) and one which attacks the tubers of the yam (*Dioscorea batatas*).

WEEVILS WHICH AFFECT IRISH POTATO TUBERS

The native home of the Irish potato is the west coast of South America, and here we find that the crop has a series of characteristic pests which may be easily disseminated in shipments of potatoes. As stated above, the writer has described three of these species in a previous paper. The description of the larva of one of them is now added and a new species described. In order that these weevils may be easily distinguished one from another by the man in the field the following table has been constructed:

TABLE OF IRISH POTATO TUBER WEEVILS

1. Prosternum grooved for reception of beak; mandibles without deciduous piece; pronotum with a deep median furrow widened angularly at middle and also behind..*Rhigopsidius tucumanus* Heller.
   Prosternum not grooved for reception of beak; mandibles with deciduous piece.. 2
2. Mandibles with tooth beneath; scrobes abruptly and broadly terminated, not extending beneath.. 3
   Mandibles without tooth beneath; scrobes narrowing and extending beneath; prothorax not as wide as elytra, angulate and broadest in front of base............ *Premnotrypes solani* Pierce.
3. Prothorax broader than elytra, subquadrate, with sides parallel to apical third, thence strongly narrowed; sides of elytra smooth..*Trypopremnon latithorax* Pierce.
   Prothorax acutely angulate at sides, widest before base; sides of elytra tuberculate, *Trypopremnon sanfordi*, n. sp.

[1] Pierce, W. D. NEW POTATO WEEVILS FROM ANDEAN SOUTH AMERICA. *In* Jour. Agr. Research, v. 1, no. 4, p. 347–352, 3 fig., pl. 39–41. 1914.

FAMILY PSALIDURIDAE PIERCE (1914)

Rhigopsidius tucumanus Heller (1906)

This weevil has been recorded by the writer [1] from Tucuman, Argentina; Cuzco, Temuco, and Arequipa, Peru; Oruro, Bolivia; and Ancud or San Carlos and Castro Islands, Chile. The weevil belongs to the subfamily Rhytirhininae and the tribe Rhytirhinini.

FAMILY PSALLIDIIDAE PIERCE (1916)

Premnotrypes solani Pierce (1914) [1]

This weevil was described from the mountain districts of Peru. It belongs to the subfamily Entiminae and the tribe Ophryastini.

Trypopremnon latithorax Pierce (1914) [1] (Pl. 29, 30)

This weevil was described from Cuzco, Peru. It belongs to the same tribe as Premnotrypes. The ventral tooth of the mandible does not belong to the deciduous piece as stated in the original description.

On June 11, 1914, Mr. H. L. Sanford found several larvæ of this species in potatoes from La Paz, Bolivia, collected by Mr. H. T. Knowles, under Federal Horticultural Board No. 2475. On June 20–26 pupæ were noticed, and an adult emerged on June 26.

This enables the writer to describe these stages.

LARVA (Pl. 29).—Length 12.5 mm. when crawling, 10 mm. when slightly curved. It is typically rhynchophorid in form, white, with light reddish brown head and dark mandibles. The essential diagnostic characters are illustrated by the author in Plate 29.

From the base of the head a median pale line passes forward. This is the epicranial suture. It divides behind the frons and forms the two frontal sutures. The frons is subtriangular, rounded behind and margined in front by the epistoma. The epicranial areas are the two large areas at each side of the epicranial suture, further bounded by the frontal suture, the pleurostoma, and the hypostoma. In front of the frons is the clypeus, and in front of this is the labrum. The clypeus and labrum partly overlap the mandibles which arise at the side of the clypeus based on the pleurostoma. Below the hypostoma at the sides of the mandibles arise the maxillæ, of which the cardo is a very large basal area. Located on the median line below the mouth opening, which is covered by labrum and mandibles, is a shield-shaped area known as *stipes labii*. Below and around this is the large basal area consisting of mentum and submentum.

There is a small abortive branch of the frontal suture extending back on the epicranium, on each side of and not far from the epicranial suture. It is terminated by a setigerous puncture. On the epicranium there are setæ arranged as follows on each lobe: One at terminus of branch of frontal suture, one on the frontal suture, two opposite middle of frons, one basal, two discal, one opposite base of mandible, two on hypostoma. On the frons there are three pairs of setæ, the two posterior pairs being about equidistant, the anterior close to the antennæ. At base of clypeus there are four tiny hairs. On labrum are four subbasal, six subapical, and six marginal hairs. The mandibles have one hair each. The maxillæ are provided with two-jointed palpi (Pl. 29, F), and a very broad setose lacinia, two setæ near base of palpi and one near base. Some of the hairs are clavate as shown in the illustration, but this is not always true, some specimens having normal hairs. The *stipes labii* has one pair of hairs. Each lobe of the mentum has one pair.

[1] PIERCE, W. D. Op. cit.

The pronotum is simple, undivided; the mesonotum and metanotum are composed of præscutum and scutoscutellum. The first six abdominal sclerites are composed of a spindle-shaped præscutum, a transverse scutum terminated by the spiracles, a spindle-shaped scutellum, and a transverse postscutellum very greatly narrowed on the dorsum. The præscutum has a few hairs. The scutellum has a row of hairs. Just above each spiracle is a tiny hair. On each epipleural lobe beneath the spiracles there is one hair. There are eight abdominal spiracles and one on the mesothorax. The seventh and eighth segments are more crowded than the preceding. The ninth and tenth are small and reduced.

PUPA (Pl. 30).—Length 10 mm., white. The most interesting features of this pupa are the rudimentary wing pads seen only when the elytra are spread. The elytral pads are not as large as often found in weevil pupæ. The antennæ are not geniculate. The beak is short. There are five pairs of hairs located on the head and beak as illustrated. On the thorax, which is subquadrate with truncate angles, there are setigerous tubercles as follows: Four on anterior margin, two antemedian and two postmedian on the disc, two pairs of antemedian and two pairs of postmedian on lateral margin. Mesonotum and metanotum with one pair of setæ each. The first abdominal segment has two pairs of setæ, and the remaining segments have a long line of setigerous tubercles. Each femur has two apical hairs, and a few ventral hairs are found as illustrated. It is interesting to note that the processes of the ninth segment are acute but reduced almost to the size of the tubercles. The tenth segment is ventral to the ninth.

Trypopremnon sanfordi, n. sp. (Pl. 28)

Described from a single specimen collected in quarantine by Mr. H. L. Sanford September 24, 1915, from a potato tuber sent by Mr. O. F. Cook from Cuzco, Peru.[1] The excellent illustrations of the type (Pl. 28) were made under the writer's supervision by Mr. H. B. Bradford.

Length 8 mm., greatest breadth 4.5 mm. Beak longer than head and narrower than eyes; the dorsal squamose portion being gradually narrowed from eyes to nasal plate. Alæ strongly flared, making the scrobes open above. Head tumid above the eyes. Median line slightly depressed on head, strongly in frontal fovea, and very faintly on beak except just behind nasal plate. Lateral depressions on beak strong. Apex of beak brownish black, with nasal plate polished, convexly raised around margin, emarginate at apex. Mandibles shining brownish black; deciduous piece reddish brown, lightest at tip, moderately long, arcuate, with sharp edges; the ventral tooth is not as acute as in *T. latithorax*; there is a slight denticle on the right deciduous piece, and the left mandible is denticulate as shown in the figure. The antennal scrobes are strongly flexed downward, very much broadened and evanescent behind; scape clavate; funicle with first two joints elongate, the others progressively shorter, the last moniliform; club as long as the four preceding joints. Head, beak, and scape densely clothed with fine silky-bronze scales, and with scattered white setæ; funicle sparsely setose; club minutely pubescent, sparsely setose.

Prothorax basally truncate, slightly broadly emarginate at middle; apically sinuate; with very strong supraocular lobes, which have vibrissæ on the inner surface; surface coarsely irregularly punctured, finely densely squamose with golden metallic scales, sparsely setose with white curved setæ; surface very uneven, with median depression bordered by antemedian ridges and two postmedian tubercles; sides prominently produced by two angulate tubercles; widest at posterior tubercles.

Elytra at base narrower than thorax; humeri tuberculate; sides subparallel but very roughly tuberculate, abruptly narrowed at posterior declivity which is nearly perpendicular. Scutellum triangular. Surface densely minutely squamose, sparsely

[1] Recorded under Federal Horticultural Board No. 4348.

setose; striæ irregular, with strong isolated punctures; entire surface covered with tubercles which are largest on the third, fifth, and seventh intervals.

Front coxæ contiguous. Prosternum strongly arcuately emarginate. Mesosternal coxæ narrowly separated. Intercoxal piece of first abdominal segment broad and deeply punctate. Second segment as long as third and fourth together.

Type.—Cat. No. 21613, United States National Museum.

WEEVILS WHICH AFFECT SWEET-POTATO TUBERS

At least four species of weevils attack the tubers of the sweet potato—namely, *Euscepes batatae* Waterhouse, *Cylas formicarius* Fabricius, *C. turcipennis* Boheman, and *C. femoralis* Faust. The adults of Euscepes and Cylas can not be confused. Those of Cylas are differentiated as shown in the "Table of sweet-potato weevils of the genus Cylas," p. 605. The pupæ of Cylas can be recognized from the fact that the direction of the appendages is anteriad, while in Euscepes it is posteriad. Reference to the illustrations will be of great assistance in separating them. The larvæ can not be so easily distinguished, as both are of the same general shape. It will be noticed, however, that they have quite a different system of abdominal folds. The larva of Euscepes is more compact. That of Cylas, when alive, is often attenuate and tightly drawn so that no folds can be distinguished. When killed in a liquid which shrinks it slightly, however, it will be noticed that the præscutal areas are proportionately larger and often subdivided transversely. The præscutum of Euscepes is not subdivided. This sclerite is the anterior sclerite of a segment and almost always has a few tiny hairs. The only other dorsal sclerite with hairs is the scutellum. In Cylas the scutellum adjoins the præscutum, and the scutum is only lateral. In Euscepes the scutellum is separated from the præscutum by the scutum.

FAMILY APIONIDAE LE CONTE (1876)

SUBFAMILY CYLADINAE PIERCE (1916)

GENUS CYLAS LATREILLE (1802)

Cylas Latreille, 1802, Hist. Nat. Gen. et Part. Crust. et Insects, t. 3, p. 196.

Type.—*Cylas brunneus* Fabricius, monotypic.

This genus contains twenty named species, of which two are widely known under the names *formicarius* and *turcipennis*. There is considerable confusion about these two species, due in part to the claims of Le Conte and Faust that they are synonymous. Fabricius described a piceous-brown Indian species with reddish thorax as *formicarius;* Olivier illustrated the species as almost pink but described it as brownish; Schönherr cited it as piceous; Gyllenhal described a species from Java with greenish-blue elytra, red thorax, and black head as *turcipennis;* Labram and Imhoff illustrated a blue species under this name. Finally Wagner presented an illustration of a species with green elytron as *turcipennis*, and he probably is right. The bluish species was named *elegantulus* by Summers. It is a common sweet-potato weevil. If it

should prove to be a variety of *formicarius* as here treated, after examination of the type, it must still be considered very distinct specifically from *turcipennis*. The National Museum collection contains six species. Sketches have been made of the side view of the head and thorax of the three species which presumably attack sweet potato, *formicarius* variety *elegantulus* (Pl. 31, A), *femoralis* (Pl. 31, F), and *turcipennis* (Pl. 31, B), and also of *brunneus* (Pl. 31, C–E), which was erroneously recorded by the writer from sweet potato in the Manual of Dangerous Insects.[1]

TABLE OF SWEET POTATO WEEVILS OF THE GENUS CYLAS

1. Male club twice as long as funicle or longer; antennæ as long as head and thorax; head not more than one-fifth shorter than beak; elytra greenish, thorax red, head black, legs red with dark band (Pl. 31, B)....... *turcipennis* Boheman.
   Male club not twice as long as funicle 2
2. Male club half to three-fourths longer than funicle, female club almost one-third shorter than funicle; male antennæ almost as long as head and thorax; head one-fourth to one-third shorter than beak; elytra bluish, thorax red, head black, legs red (Pl. 31, A)...........*formicarius* Fabricius, var. *elegantulus* Summers.
   Male club half longer than funicle; head as long as beak; antennæ as long as thorax plus head behind eye; elytra black with blue or green luster, suture piceous; thorax black, margins piceous; head black; legs dark red with black ring on femora (Pl. 31, F)...*femoralis* Faust.

Cylas formicarius Fabricius (1798)

Brentus formicarius Fabricius, 1798, Sup. Ent. Syst., p. 174, no. 5.

Fabricius[2] gave the following description:

Habitat Tranquebariae.

Parvus in hoc genere. Rostrum cylindricum, atrum, antennis rufis, moniliformibus: articulo ultimo longiori, cylindrico, clavato. Thorax rufus, antice globosus. Elytra laevia, atra, nitida. Pedes rufi, femoribus clavatis, at inermibus: annulo nigro.

In altero sexu antennarum clava brevior, ovata.

Olivier and Schönherr described the species as piceous with ferruginous thorax, antennæ, and legs. It hardly seems possible that this can be the same species as the common sweet potato weevil with shiny blue-black elytra, red thorax and appendages, and black head and beak.

For this reason it is considered best to apply to the sweet-potato weevil a name which certainly applies to it—*elegantulus* Summers. In order that economic entomologists may not be inconvenienced greatly, and in deference to the many writers who have assigned Fabricius's name to the sweet-potato weevil, *elegantulus* may be considered as a variety of *formicarius* until there can be an examination of the type.

Cylas formicarius elegantulus Summers (1875), the Sweet Potato Weevil (Pl. 31, A; Pl. 32, A, B; Pl. 33, E–H; Pl. 34, A–D)

Otidocephalus elegantulus Summers, 1875, *in* New Orleans Home Jour., Jan. and Dec.

Cylas formicarius Le Conte, 1876, *in* Proc. Amer. Phil. Soc., v. 15, p. 327. *O. elegantulus* is quoted in synonymy.

[1] PIERCE, W. D. A MANUAL OF DANGEROUS INSECTS ... p. 209. 1917. Published by the United States Department of Agriculture, Office of Secretary.

[2] FABRICIUS, J. C. SYSTEMA ELEUTHERATORUM . . . v. 2, p. 549. Kiliae, 1801.

This is the common sweet-potato weevil (Pl. 31, A; 32, A, B) with bluish elytra, red thorax and appendages, and black head. The illustration of the adult is drawn from a New Orleans specimen. The side view of head and thorax is from a Hawaiian specimen. There is quite a range of difference in measurements of the species but the analysis of these differences is reserved for a more technical paper now in preparation. The immature stages are described from specimens collected at Victoria, Texas.

LARVA. (Pl. 34, A–D).—The larva of this species measures from 5 to 8 mm. in length and is white, with light brownish head and darker brown mandibles. The head shield is slightly angulately emarginate behind. From the center of the emargination on the median line the epicranial suture passes forward, separating the epicranium into two parts; this suture divides behind the frons and forms the two frontal sutures. The frons is subtriangular, emarginate at anterior angles for antennæ, and emarginate along epistoma for attachment of clypeus. The median line is impressed and darkened. The frons has three pairs of large setæ, the posterior pair being closest and the median pair but little more separated. The anterior pair are located very close to the antennal fossæ. A tiny pair of setæ are located in such a way as almost to form an equilateral triangle with the posterior and median setæ.

The epicranial areas are located on each side of the epicranial suture. Each lobe bears the following setæ: One very close to the apex of frons, one slightly posterior to this and farther from the median line, one opposite the middle of the frons, one a little farther from the median line on the same line as the preceding, one toward the base of the frons, one opposite the middle of the mandible, one opposite the hypostomal angle of mandible, one on hypostoma near base of mandible, one opposite but distant from mandible, and three forming a triangle on disc of epicranium.

The antenna is a fleshy, two-jointed appendage located at the lateral angle of the frons. The mandibles are very bluntly bidentate. Each mandible has a tiny hair about the middle. The clypeus is attached in front of the frons and is broadly transverse. It bears on the epistomal margin four tiny hairs. The labrum is not as broad, is rounded in front, and has a row of four setæ behind the middle, a seta on each side in front and closer than the outermost setæ, and four marginal setæ. The maxillæ are elongate, terminated by a two-jointed palpus and a setose lacinia. The maxillæ are provided with four setæ, two near palpus, one at middle, and one at base. The *stipes labii* is cordate, bearing two-jointed palpi and a single pair of setæ. Each lobe of the mentum is provided with two pairs of setæ.

The entire surface of the body is covered with tiny pubescence.

The prothorax is dorsally not divided but has the præscutal and scutal areas indicated by rows of setæ. The mesothoracic spiracle is located on a lobe very close to the prothorax. The præscutum of the prothorax and that of the mesothorax are provided with a few small hairs. The scutellum is marked with a row of hairs.

The first eight abdominal segments are normal and each bears a spiracle. The præscutal area is more or less transversely divided, and its posterior lobe is marked with a few tiny setæ. The scutellum is large and prominent and provided with a row of setæ. The scutum is only lateral and has just above the spiracle a tiny seta. Each epipleural lobe is provided with a single seta, and each hypopleural lobe with two setæ. The coxal lobes of the thorax bear several setæ, and those of the abdomen a single seta each. The last two abdominal segments are simple and provided with a number of setæ.

PUPA (Pl. 33, E–H). Elongate, about 6 mm. long, white. This pupa is especially characterized by the nongeniculate antennæ which lie parallel to the legs. The antennæ and two anterior pairs of legs are directed cephalad instead of posteriad,

as in the Curculionidae. Another characteristic is that the femora and tibiæ of the posterior pair, being directed in the same manner, are completely covered by the wings. The head and beak are elongate and provided with setigerous tubercles as follows: One pair between the eyes at base, one pair immediately behind eyes, two tiny pairs between eyes, and two pairs on beak; the posterior pair being close to the eyes, and the anterior behind the middle. The antennæ are roughly tuberculate.

The prothorax is margined anteriorly by four pairs of setigerous tubercles and has one pair of discal setæ. The mesothorax has two pairs of large setigerous tubercles and a lateral pair of tiny setæ. The femora bear two or three setæ. The knees of the posterior femora are visible dorsally only.

The mesothorax bears three pairs of small setæ between the bases of the elytra. The metathorax is provided with two rows of setæ on tiny tubercles, the anterior row having two pairs and the posterior row six pairs. The abdominal segments have dorsally five pairs of setigerous tubercles near the posterior margin, a pair of tiny setæ near the middle of the segment, and lateral setigerous tubercles.

The ninth segment is provided with two large curved processes. The tenth segment is ventral to the ninth.

Cylas turcipennis Boheman (1833) (Pl. 31, B)

Cylas turcipennis Boheman, 1833, *in* Schönherr, Gen. et Spec. Curc. v. 1, p. 369-370.

The brief preliminary diagnosis of the species presented by Boheman is as follows:

Elongatus, viridi-cœrulescens, nitidus, antennis thorace pedibusque rufis, capite cruciatim impresso, rostro punctulato, elytris modice convexis, subtiliter striato-punctatis. Habitat in Java, in India orientali.

The following dimensions are included in the detailed description: Length 3 lines (6 mm.); antennæ as long as thorax and head; club of male antenna longer than preceding joints; beak not longer than head; elytra twice as wide as thorax at base, and twice longer than wide. The color description is as follows: Head obscurely viridi-cœruleous; beak almost black; antennæ rufo-ferruginous; thorax shining rufous; elytra cœrulescent-virescent; thorax beneath rufous, remainder of body beneath cœruleo-virescent; legs rufous; tarsi beneath fulvous, spongy; female with femora in middle annulate virescent.

Two specimens from Palembang, Sumatra, collected by Mr. M. Knappert, are here considered as this species. They differ only in having the beak slightly longer than the head, and a statement to this effect might have been made if the description had been based on examination with a low-power lens. Two other specimens are at hand from Bay Laguna Province, Philippine Islands, collected by Mr. P. L. Stangl. A part of a body of a weevil from Guatemala, collected by Mr. D. G. Eisen, is also undoubtedly this species. Pascoe records the species from Sarawak, Java, and India.

Cylas femoralis Faust (1898) (Pl. 31, F)

Cylas femoralis Faust, 1899, *in* Deut. Ent. Ztschr., p. 24.

This species was collected by Mr. Rolla P. Currie at Mount Coffee, Liberia, in February to April, 1897, and he has informed the writer that it was a serious sweet-potato pest in that country. It is described from Kamerun. In the Manual of Dangerous Insects [1] this species was referred to as *C. brunneus* by mistake.

[1] PIERCE, W. D. A MANUAL OF DANGEROUS INSECTS ... p. 209. 1917. Published by the United States Department of Agriculture, Office of Secretary.

FAMILY OROBITIDAE PIERCE (1916)

Subfamily Orobitinae Pierce (1916)

GENUS EUSCEPES SCHÖNHERR (1844)

Euscepes Schönherr, 1844, Gen. et Spec. Curc., v. 8, pt. 1, p. 429. Type.—*porcellus* Boh. by original designation.

Euscepes Lacordaire, 1866, Gen. Coleop., v. 7, p. 100–101. Type.—*porcellus* Boh.

Hyperomorpha Blackburn, 1885, *in* Sci. Trans. Roy. Dublin Soc., s. 2, v. 3, p. 182–183. Type.—(*squamosa*, Blackburn)=*batatae* Waterhouse.

Euscepes Champion, 1905, *in* Biol. Centr.-Amer., Coleopt., v. 4, pt. 4, p. 496–498. Type.—*porcellus* Boh.

Lacordaire caused a confusion of genera by wrongly interpreting the number of funicular joints, of which there are seven. This error was corrected by Champion. The two genera Euscepes and Hyperomorpha are strictly congeneric; in fact, the two type species differ principally in size. A large series of *porcellus* from various parts of Central America is at hand. These have been carefully compared with Blackburn's description of Hyperomorpha, but no generic difference can be found.

The rostral canal extends along the prosternum and ends in a mesosternal pocket. The beak when at rest fits tightly into this canal. The prothorax is lobed to cover the eyes when at rest. The body is elongate.

Euscepes batatae C. O. Waterhouse (1849), the Scarabee of the Sweet Potato (Pl. 32, C, D; Pl. 33, A–D; Pl. 34, E–H)

Cryptorhynchus batatae Waterhouse, 1849, *in* Trans. Ent. Soc., London, v. 5, p. LXIX.

Hyperomorpha squamosa Blackburn, 1885, *in* Sci. Trans. Roy. Dublin Soc., s. 2, v. 3, p. 182–183.

Euscepes batatae Champion, 1905, *in* Biol. Centr.-Amer., Coleopt., v. 4, pt. 4, p. 497.

This weevil (Pl. 32, C, D) is one of the most serious cosmopolitan pests of the sweet potato, although hitherto it has been recorded only from Barbados, St. Vincent, and Antigua, St. Kitts, Nevis, and Hawaii. In all of these places, however, it is reported as damaging sweet potatoes. The receipt of two specimens from Dr. Da Costa Lima, of Brazil, with the statement that they were injuring sweet potatoes at Rio de Janeiro, caused the writer to make a search through the undetermined collections of the National Museum with the result that the known distribution of the species is hereby greatly extended. Specimens are at hand from Barbados, injuring sweet potatoes May 22, 1900, and more recent material; Hope, Kingston, Jamaica, on sweet potatoes, Mr. S. F. Ashby; Campinas, Brazil, injuring sweet potatoes, August, 1913, Mr. A. Hempel (No. 100); Rio de Janeiro, Brazil, injuring sweet potatoes, July, 1917, Carlos Moreira; Honolulu, Oahu, Hawaii, bred from sweet potato; Kaimuki, Oahu, Hawaii, bred from sweet potato; Guam, on sweet potato, Mr. D. T. Fullaway; Norfolk Island, New Zealand, March, 1883, Mr. P. H. Metcalfe; Mayaguez, Porto Rico, injuring sweet potatoes, 1912, 1914, 1917, Mr. C. W. Hooker, Mr. R. H. Van Zwaluwenburg.

This extensive distribution indicates that there are probably many other countries where sweet potatoes are grown that may have the weevil. If, fortunately, it should prove to be absent in other countries, rigid quarantines should be put into effect, such as that recently estab-

lished by the United States. In fact it has been only because of the excellent system of quarantine inspection in California that the species has not already come into the United States with Hawaiian potatoes. Many shipments of infested sweet potatoes have already been intercepted at the California ports.[1]

The following is a description of the species redrawn to include all the material at hand. The variations of color will be mentioned in subsequent paragraphs.

Length about 4 mm. Brown, mottled with lighter areas, especially by a transverse, irregular, postmedian band on the elytra. Squamose, bristling with upright setæ. Beak curved, carinate and laterally bifurcate. Front foveate. Front and beak bristling with erect scales. Vertex provided with more decumbent scales. Prothorax constricted in front, laterally impressed on disk behind, mottled with erect scales, except on posterior margin which is provided with smaller, more decumbent scales. Elytral striæ composed of rather distant punctures, each bearing a small scale; surface closely set with overlapping scales and each interspace with a single series of elongate squamiform setæ. Undersides more sparsely clad with semierect scales. Legs provided with scales and setæ. Rostral canal deep, terminating in a prominent pocket of the mesosternum. Intercoxal process broad, angulate on anterior margin. First segment behind coxæ subequal to the second, which is but slightly longer than the subequal third and fourth segments. The femora are minutely toothed.

Mr. Bradford's excellent illustrations will be very helpful in identifying this weevil. His illustration of the adult is from Hawaiian material.

The species varies from very light brown to almost black and on the darkest specimens the mottling and the postmedian vitta have practically disappeared.

On light specimens the scales of the thorax are mostly dark brown, with flecks of pale scales and with the basal scales orange colored. The scales of the elytra are mottled in many shades of brown. The postmedian fascia extends to the fifth interspace and is bordered by very dark scales and divided by a wavy dark line. The erect setæ are mixed dark and white. The ventral scales are pale, but on the legs they are mottled dark brown and pale. This description fits best some of the Jamaican, Barbados, and Brazilian specimens. Almost black material comes from Brazil, Jamaica, and Guam. The Hawaiian specimens are a duller brown, and the New Zealand material is the lightest of all. There is, however, no doubt of the specific identity of the entire series.

In order that the immature stages may be readily distinguished from those of *Cylas formicarius* a series of very careful drawings of the essential characters of the larva and pupa have been made by Mr. Harry Bradford under the writer's direction. The drawings of the vertex and face are by the writer. Barbados material was used for these drawings.

LARVA (Pl. 34, E–H). The larva of this species measures about 5 mm. in length and is white, with a yellowish head and reddish brown mandibles tipped with black. The maxillæ and labium are slightly tinged with brown.

The head shield is broadly, angulately emarginate behind; from the center of the emargination on the median line the epicranial suture passes forward, separating the epicranium into two parts. This suture divides behind the frons and forms the two

[1] WHITNEY, L. A. THE SMALL SWEET POTATO WEEVIL (CRYPTORHYNCHUS BATATAE WATERH.). *In* Mo Bul. State Com. Hort. [Cal.], v. 4, no. 3, p. 162-164, fig. 24–28. 1915.

frontal sutures. The frons is subtriangular, rounded at anterior angles, and slightly emarginate for antennæ; its front margin is the epistoma. There are three pairs of large setæ on the frons, the posterior pair being located rather close together and near the apex of the triangle. The second pair are farther apart and halfway to the front. The anterior pair are located near the lateral angles just behind the antennæ. A tiny pair of setæ are located just in front of the posterior pair of large setæ. A tiny pair are located slightly behind and outside of the median pair of large setæ. The median line of the frons is impressed from the posterior angle almost to the middle.

The epicranial areas are the two large areas on each side of the epicranial suture further bounded by the frontal suture, the pleurostoma, and the hypostoma. The following setæ occur on each lobe of the epicranium: One opposite the apex of the frons, one on the disk of the epicranium in the line with the preceding; one opposite the middle of the frons and near the frontal suture; one near the hypostoma toward the base of the mandible; one as close to the hypostoma and opposite the base of the maxilla; one near the pleurostoma; the last three forming a triangle. Forming a semicircular line with the setæ opposite the frons are a tiny seta, a longer one opposite the antenna, and a long one opposite the pleurostomal seta.

The antenna is a small, fleshy, two-jointed appendage at the angle of the frontal suture and pleurostoma. The mandibles are bluntly bidentate and have two small setæ. The clypeus is attached in front of the frons and is broadly transverse. It bears on the epistomal margin four tiny hairs. The labrum is not as broad; it has a pair of median setæ and three pairs of marginal setæ. The maxillæ attached at the side of the mandibles are terminated by a two-jointed palpus and a setose lacinia. The maxillæ are provided with three setæ, two near the palpi and one toward the base. The *stipes labii* is appendiculate, bilobed, bearing two-jointed palpi and a single pair of posterior setæ. Each lobe of the mentum is provided with one seta. The thoracic segments are simple, being composed of only præscutum and scutoscutellum. In the prothorax there is no separation of these parts, but they are indicated by the arrangement of the setæ. The abdominal segments are dorsally composed of four single sclerites, namely, præscutum, scutum, scutellum, and postscutellum.

The thoracic spiracle is located on a lobe of the mesothorax, very close to the head. The abdominal spiracles are located on a small lobe at the side of the scutum on the first eight segments. The ninth and tenth abdominal segments are smaller and considerably modified.

Setæ are arranged as follows: Four pairs of tiny hairs on anterior margin of prothoracic præscutal area; a row of longer hairs on the scutal area of the prothorax; each segment from the mesothorax back with two pairs of long lateral setæ, near which are located smaller and inconspicuous setæ; each præscutum with a single pair of setæ; on each segment of the abdomen the lateral lobe of the scutellum is provided with a tiny seta, behind and close to the spiracle; each epipleural lobe below the spiracle with two setæ; each coxal lobe with several setæ which are more conspicuous on the prothorax.

Pupa (Pl. 33, A–D).—Length 4 mm., white. This is a normal, characteristic curculionid pupa with geniculate antennæ and legs turned posteriad. Head oval, beak short. The head bears four pairs of basal setigerous tubercles, two pairs of interocular tubercles, and one pair of tiny setæ at base of beak. Prothorax with two pairs of antero-marginal setigerous tubercles; one pair of antero-lateral and one pair of postero-lateral setigerous tubercles; four pairs of dorsal tubercles and one pair of ventral. The femora are apically armed with two setæ. Mesonotum and metanotum each provided with two pairs of setæ. Each abdominal segment bears four dorsal and one or more lateral setæ. The ninth segment is armed with two very large processes. The tenth segment is very small and located on the venter of the ninth.

A WEEVIL WHICH ATTACKS THE TUBERS OF YAMS

Palaeopus dioscoreae, n. sp. (Pl. 32, E, F)

Described from two specimens reared from tubers of *Dioscorea batatas*, Hope, Kingston, Jamaica, by Mr. S. F. Ashby in April, 1914. Belongs in the same subfamily as Euscepes.

Length 4.5 mm., breadth 1.75 mm. Piceous black, with reddish brown appendages. It is sparsely clad with dark brown or whitish oblong decumbent scales and erect, longer truncate scales of variable color. The punctuation is very coarse.

Head smooth, beak separated from head by strong transverse constriction. Beak longitudinally five-striate and bristling in basal half with erect brown scales; apical half smoother, with confluent punctures and flat scales. Scrobes beginning beyond middle, diagonal, reaching eyes beneath. Eyes lateral, separated by width of beak, covered when at rest by pronotal lobes. Antennal funicle seven-jointed, first joint a little longer than second. Prothorax broad, depressed, convexly rounded on sides, bisinuate at base, lobed over eyes, truncate at apex which is about half as wide as base; median line broadly elevated; punctuation very coarse. Elytra 10-striate, the tenth striæ abbreviated; strial punctures large, rounded, well separated, and setigerous; interspaces not wider than striæ, clad with a single row of erect squamose setæ of variable color. Scutellum indistinct. Elytra broader at base than thorax; with distinct humeri.

Sternal canal deep, sharply margined, limited by a cuplike depression of the mesosternum. Posterior coxæ very broadly separated. Metasternum at middle as long as first abdominal segment behind coxæ. Intercoxal piece of first abdominal segment angulate at middle. Second segment not as long as third and fourth together.

Femora dentate and canaliculate beneath. Tibiæ curved at base, strongly hooked at the apex. Tarsal claws simple.

Type.—Cat. No. 21612, United States National Museum.

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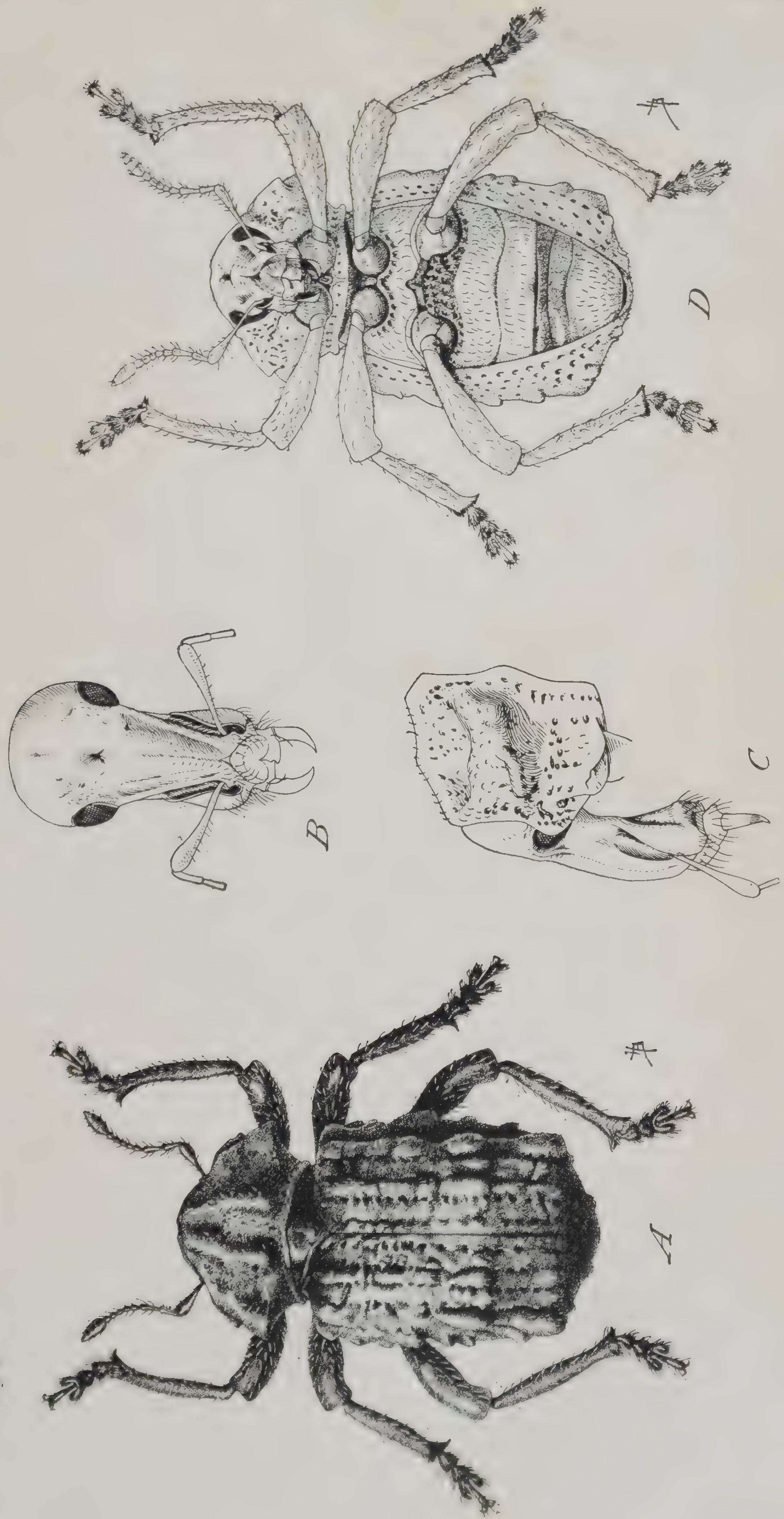
PLATE 28

Trypopremnon sanfordi: Adult from Cuzco, Peru

A.—Dorsal view. Actual length 8.025 mm.
B.—Face of same. Actual length of head and beak 3.5 mm.
C.—Side view of thorax and head.
D.—Ventral view of adult.
Drawn by Mr. H. B. Bradford.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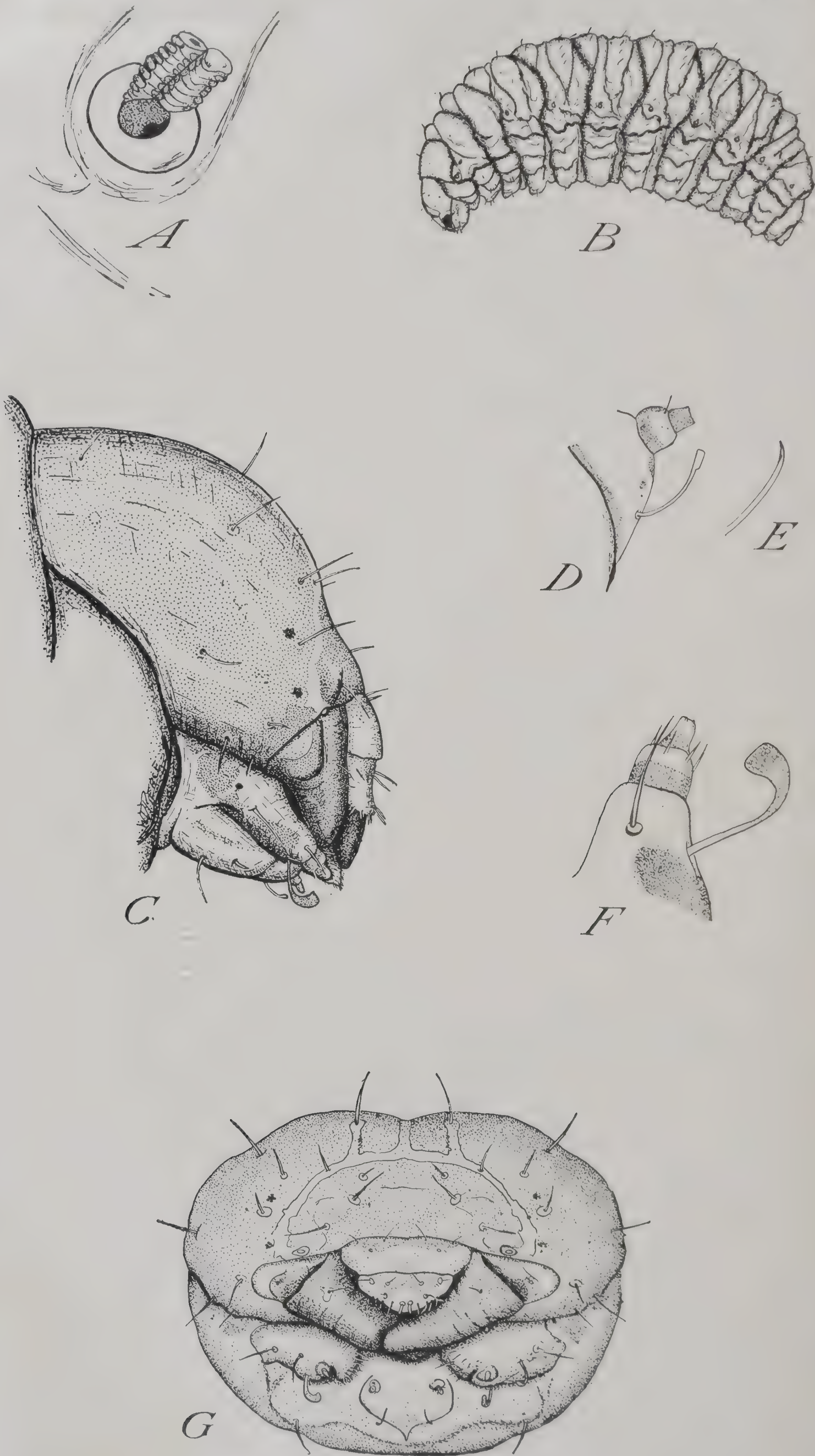
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PLATE 29

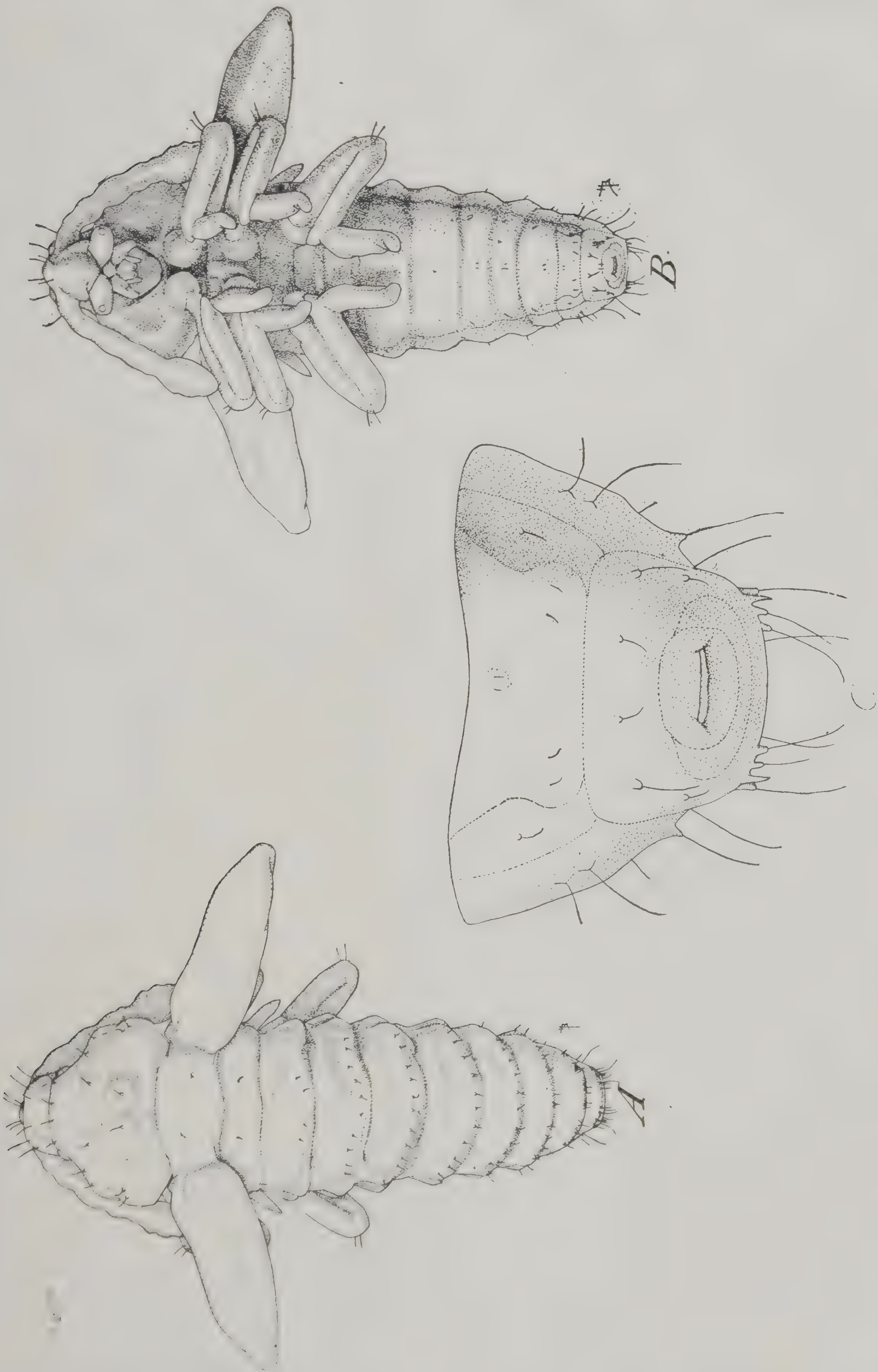
Trypopremnon latithorax: Larva from La Paz, Bolivia

A.—Prothoracic spiracle.
B.—Larva, lateral view.
C.—Lateral view of head.
D.—Right side view of apex of labium.
E.—Corresponding hair on left side.
F.—Maxillary palpiger and palpus, lateral view.
G.—Face.
Drawn by the author.

PLATE 30

Trypopremnon latithorax: Pupa from La Paz, Bolivia

A.—Dorsal view.
B.—Ventral view.
C.—Enlarged sketch of eighth, ninth, and tenth abdominal segments.
Drawn by Mr. H. B. Bradford.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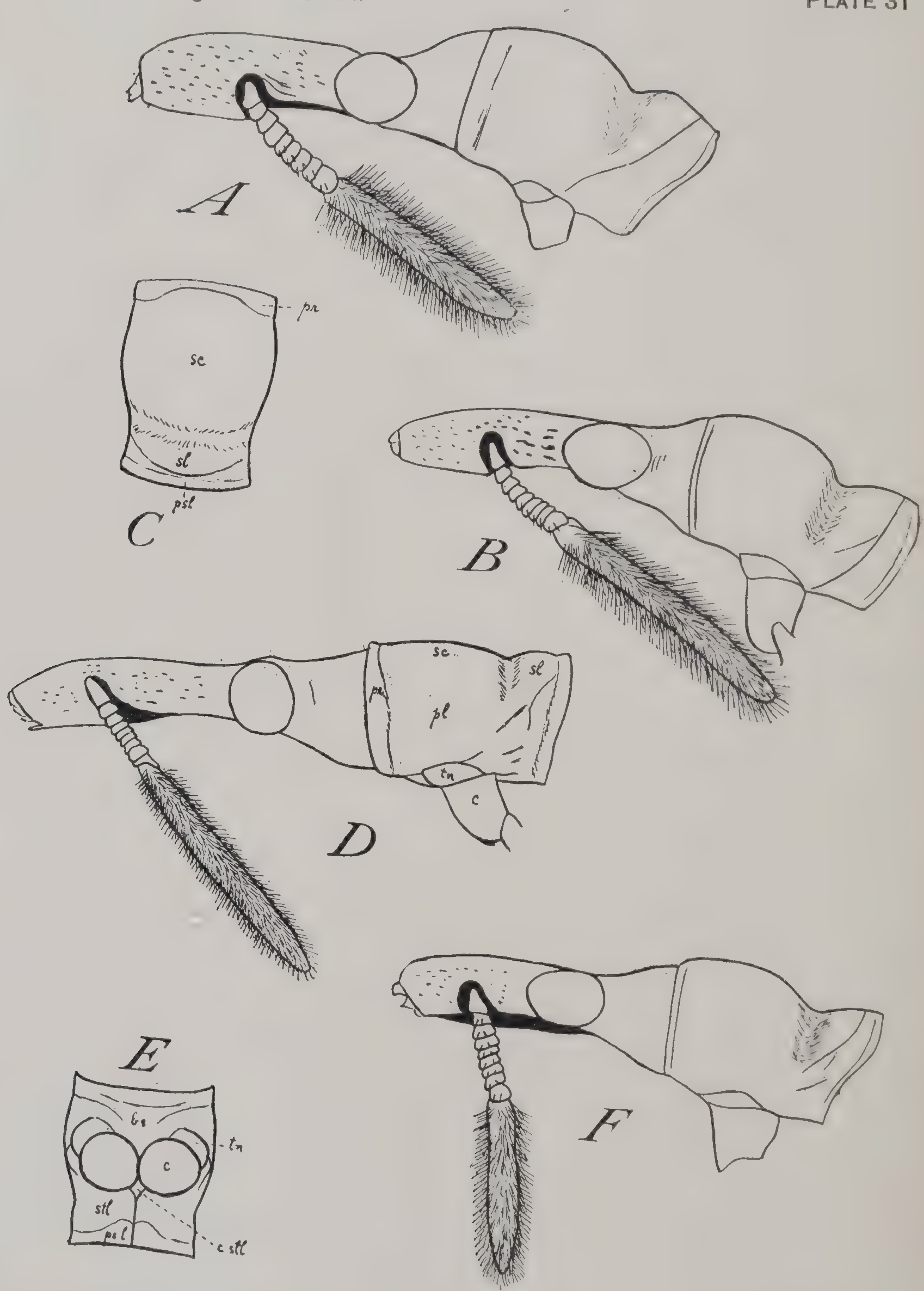
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PLATE 31

Species of the genus Cylas:

A.—*Cylas formicarius elegantulus* from Honolulu, Hawaii, side view of head and thorax.

B.—*Cylas turcipennis* from Sumatra, side view of head and thorax.

C.—*Cylas brunneus* from East Africa, dorsal view of thorax.

D.—*Cylas brunneus*, side view of head and thorax.

E.—*Cylas brunneus*, ventral view of thorax.

F.—*Cylas femoralis*, side view of head and thorax.

The abbreviations used on this plate are as follows: *pr*, Presegmental ring; *sc*, scutum; *sl*, scutellum; *psl*, postsegmental ring; *pl*, pleurum; *tn*, trochantin; *c*, coxa; *bs*, basisternite; *stl*, sternellum; *cstl*, centrosternellum.

Drawn by the author.

PLATE 32

Sweet-potato and yam weevils:

A.—*Cylas formicarius elegantulus*, female, from sweet potatoes, New Orleans, La.
B.—Same, head of male.
C.—*Euscepes batatae*, from sweet potatoes, Hawaii.
D.—Same, side view of head.
E.—*Palaeopus dioscoreae*, from yams (*Dioscorea batatas*), Jamaica.
F.—Same, side view of head.
Drawn by Mr. H. B. Bradford.

PLATE 33

Pupæ of sweet-potato weevils:

A.—*Euscepes batatae,* Barbados, venter (length 4 mm.).

B.—Same, latero-ventral view of fifth to tenth segments.

C.—Same, dorsal view.

D.—Same, venter of seventh to tenth segments (length of this portion 1 mm.).

E.—*Cylas formicarius elegantulus*, Victoria, Texas, ventral view of sixth to tenth segments (length of this portion 1 mm.).

F.—Same, ventral view (length 6 mm.).

G.—Same, latero-ventral view.

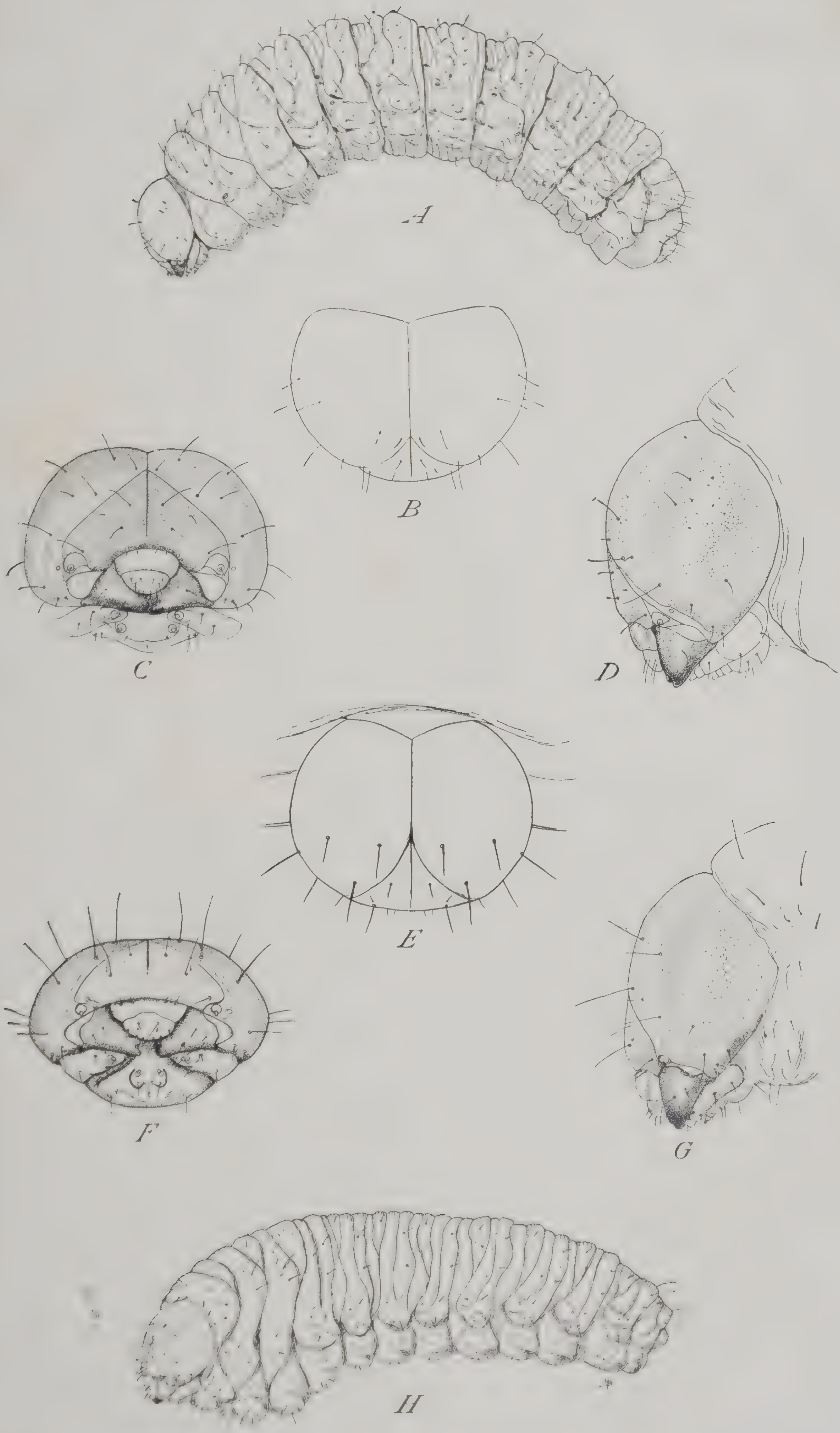
H.—Same, dorsal view.

Drawn by Mr. H. B. Bradford.

PLATE 34

Larvæ of sweet-potato weevils:

A.—*Cylas formicarius elegantulus*, Victoria, Texas, lateral view.
B.—Same, dorsum of head.
C.—Same, face.
D.—Same, side of head.
E.—*Euscepes batatae*, Barbados, dorsum of head.
F.—Same, face.
G.—Same, side of head.
H.—Same, lateral view of larva.
Drawn by Mr. H. B. Bradford.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JOURNAL OF AGRICULTURAL RESEARCH

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STERILITY IN THE STRAWBERRY[1]

By W. D. VALLEAU

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INTRODUCTION

This paper is a report of studies on the sex condition in strawberries (*Fragaria* spp.) which have been carried on during the past four years. The study of pistil sterility and anther abortion in the cultivated varieties and wild species, which are the result of the strong tendency of this genus toward dieciousness, has received considerable attention; but the primary object of the investigation has been to find, if possible, some satisfactory explanation for the phenomenon of pollen abortion, which is so prevalent among heterozygous plants or plants of hybrid origin.[2]

MORPHOLOGY OF THE FLOWER PARTS

INFLORESCENCE

The inflorescence of our cultivated strawberry and of *Fragaria virginiana*, which it closely resembles, is a dichasial cyme or sometimes, especially in certain cultivated varieties, a pleiochasium. The two lateral branches of the relatively main axis are not always equal either in size—that is, number of flowers borne—or in time of flowering. The pedicel of the primary flower is generally inserted a short distance from the joint of the two secondary branches and on the smaller of the two. The primary flower of the largest lateral branch usually opens directly after the primary flower and before that of the smaller lateral branch. The arrangement of the flowers and order of blooming are shown in figure 1.

Variations from these types are not uncommon. In some cases the primary flower is lacking; in others, the primary stalk seems to be made

[1] Published, with the approval of the Director, as Paper 94 of the Journal Series of the Minnesota Agricultural Experiment Station.

[2] The work was begun at the suggestion of Dr. M. J. Dorsey, of the Minnesota Agricultural Experiment Station, and I wish to express my thanks for the help and encouragement which he gave during the progress of the work. I also wish to express my appreciation of the assistance given by Dr. C. E. Allen, of the Department of Botany, University of Wisconsin, in a portion of the cytological studies and for space kindly furnished me in his laboratory during a month in 1915.

up of two which separate at varying distances from the ground, thus producing two typical cymes from one main stalk. The peduncle and pedicel lengths vary greatly within a variety, but there is apparently a rather constant negative correlation between the two—that is, as the peduncle or primary stalk decreases in length the pedicels or secondary branches increase, resulting in a rather constant ratio between fruit stalk length and leaf petiole length or height of plant.

Opposite the point of insertion of the small secondary branch is usually a large bract. This may be and usually is in the wild forms a monophyllous leaf, while in many of the cultivated varieties it may be a well-developed di- or tri-phyllous leaf. The bracts or bracteoles subtending the branches of lesser degree are usually only rudimentary structures, being made up of the stipules often much reduced, but with an occasional slight broadening of the midvein to form a small leaf blade.

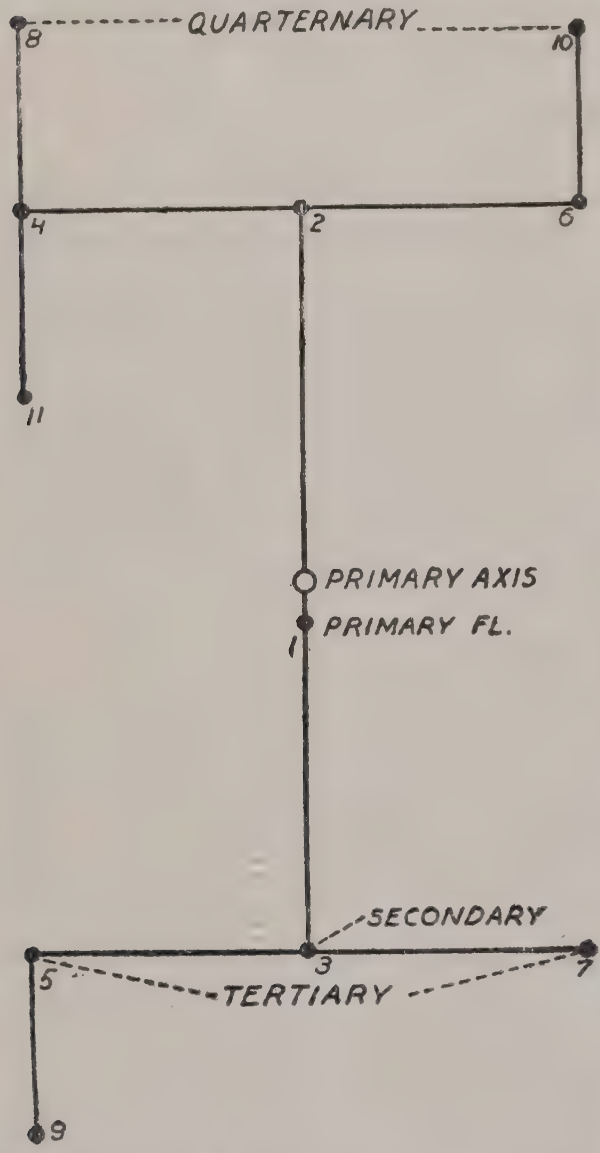

FIG. 1.—Diagram showing the arrangement of flowers of the strawberry and the order of blossoming. The approximate order of opening is indicated by the figures.

The flowers are hypogynous, and typically pentamerous with regard to all parts except the carpels. The perianth consists of three whorls of members, the outer five epicalyx lobes alternating with the five sepals and opposite to the five white petals.

STAMENS

STAMEN ARRANGEMENT.—The stamens in typical flowers are arranged in multiples of five. The number is not constant in pure species or within a variety. The stamens are arranged in three whorls. The outer consists of 10 parapetalous stamens located at either side of the base of the petals (fig. 2, *a*, *PP*). These have long filaments. Their number is the most constant of any of the whorls. The second whorl consists of five antipetalous stamens located opposite the petals and inside of the parapetalous whorl (fig. 2, *a*, *AP*). The filaments are shorter than those of the outer and inner whorls. The third whorl consists of five antisepalous stamens inserted opposite the point of insertion of the sepals, and inside of the two other whorls (fig. 2, *a*, *AS*).

Variations in stamen number from the above arrangement, if slight, are usually due to the addition or loss of one or more stamens from the antipetalous series. If a definite increase of five takes place, it may be the result of an increase in one of two places: either the 5 single antipetalous stamens may be replaced by 5 pairs to form a whorl of 10 (fig. 2, *b*, *AP*), or the 5 antisepalous stamens may have been replaced by 5 pairs of parasepalous stamens located at the same points as the whorl of 5 (fig. 2, *c*, *PS*). A further increase in the number of antipetalous stamens may consist in the development of a pair located on either side and slightly inside of the single antisepalous stamens (fig. 2, *d*, *AP*). These are characterized by the same short filament that is found in the anti-

Fig. 2.—Flower diagrams of *Fragaria* spp., showing stamen arrangement: *AS* represents antisepalous; *AP*, antipetalous; *PP*, parapetalous; and *PS*, parasepalous stamens. *a* represents the 20-stamen arrangement found commonly in *F. virginiana* and *F. americana* and many cultivated varieties; *b* and *c*, a 25-stamen arrangement found in *F. virginiana* and some cultivated varieties; *d*, a 35-stamen arrangement sometimes found in cultivated varieties; while *e* and *f* represent a 10 and 15 stamen arrangement found in some clones of *F. americana*.

petalous whorl of 5. This increase, plus the 20-stamen arrangement, gives a 30-stamen arrangement, or with either of the two 25-stamen arrangements, gives 35.

Rydberg (*34, p. 10*)[1] has pointed out that the antipetalous stamens which appear to be the middle whorl in *Fragaria* spp. are truly an inner whorl which has been pushed out to form apparently a middle one (fig. 2, *d*), and that the outer parapetalous stamens are in reality younger with regard to development than the antisepalous or inner series. A study of the position of the accessory stamens of the antipetalous series (which can readily be distinguished by their short filament) and of the order of stamen development indicates that this view is correct.

[1] Reference is made by number (italic) to "Literature cited," p. 666–669.

In *F. americana*, 10, 15, and 20-stamen arrangements are common. The 20-stamen arrangement is the same as that described for *F. virginiana* and the cultivated varieties. When, however, a decrease below 20 to 15 takes place, it is due to the loss of the short filamented middle whorl of antipetalous stamens (fig. 2, *f*)—further proof that this is truly an inner and not a middle series. The next series to be lost is that consisting of the inner long filamented antisepalous stamens, thus leaving the parapetalous stamen arrangement (fig. 2, *e*). This seems peculiar, in view of the fact that they are older than the parapetalous stamens and therefore should remain longest. This might be considered as proof that—

the parapetalous stamens must be regarded as abnormal supernumary parts,

as Rydberg (*34, p. 11*) considers them. The genus Fragaria differs from other species of the Potentilleae in this respect, as the more usual order of loss is first, the parapetalous, followed by the antipetalous stamens (*34, p. 11*), the long filamented antisepalous stamens being the most permanent. When stamens are dropped in *Fragaria* spp., they are lost completely and do not form the staminoids or partially developed stamens which are found in the pistillate flowers, so that a decrease in stamen number can not be considered as a step toward dieciousness.

Staminodia.—Typically *F. virginiana* and, as will be shown later, some other species of strawberry are diecious, although the separation into strictly staminate and pistillate forms is not complete. The flowers of pistillate plants bear staminodia showing varying degrees of development (fig. 3, *11–16*), which never, as far as I have observed, produce pollen.[1] The staminate plants develop pistils which, as far as outward appearances are concerned, are normal, but which do not set fruit. As a result of this incomplete separation of the sex-bearing organs, there are variations in the stamen and pistil condition on individual clones and also on the flowers of an infloresence within a clone.

The most common condition in the staminate plants is that in which all of the flowers produce normal stamens bearing good pollen. Occasionally clones are found in which the first flower bears only staminodia in place of the normal stamens. In flowers of this type the pistils are practically always fertile and produce normal fruits. On the other hand, the primary flower may produce normal stamens and no fertile pistils while one or both of the secondary flowers may be of the pistillate

[1] A possible case of pollen production in a pistillate variety is that of the Crescent. Plants of it which I have observed are strictly pistillate, although producing very large prominent staminoids (fig. 3, *16*)-which are entirely devoid of reproductive tissue (Pl. 36, A). Castle (*7, p. 150*) states that in England "it produces perfect flowers and sets its fruit most readily, cropping heavily in favorable seasons." As most other English varieties are hermaphrodites, it is very possible that large crops might set as a result of cross-pollination, and that the presence of the extremely large staminoids has been misleading with regard to the exact sexual condition of these plants.

Fletcher (*15, p. 132*) also states that Crescent may vary in stamen condition becoming "a true stami, nate on rich soils," but gives no further evidence on the point.

type, in which case they set fruit. A few cases have been found in which one side of a flower produced normal stamens and sterile pistils while the other half produced staminodia and set fruit. A similar range of conditions with regard to stamens has been noticed in seedlings of certain cultivated varieties. Figures G and H, Plate 35, are from photographs of primary and secondary flowers of the seedling Minnesota 1017 × Progressive 32–1, both of which bear only staminoids, while I and J represent secondary and tertiary flowers of the same variety, I producing both staminodia and normal anthers and J producing only normal anthers. The production of pistillate flowers on the primary and on

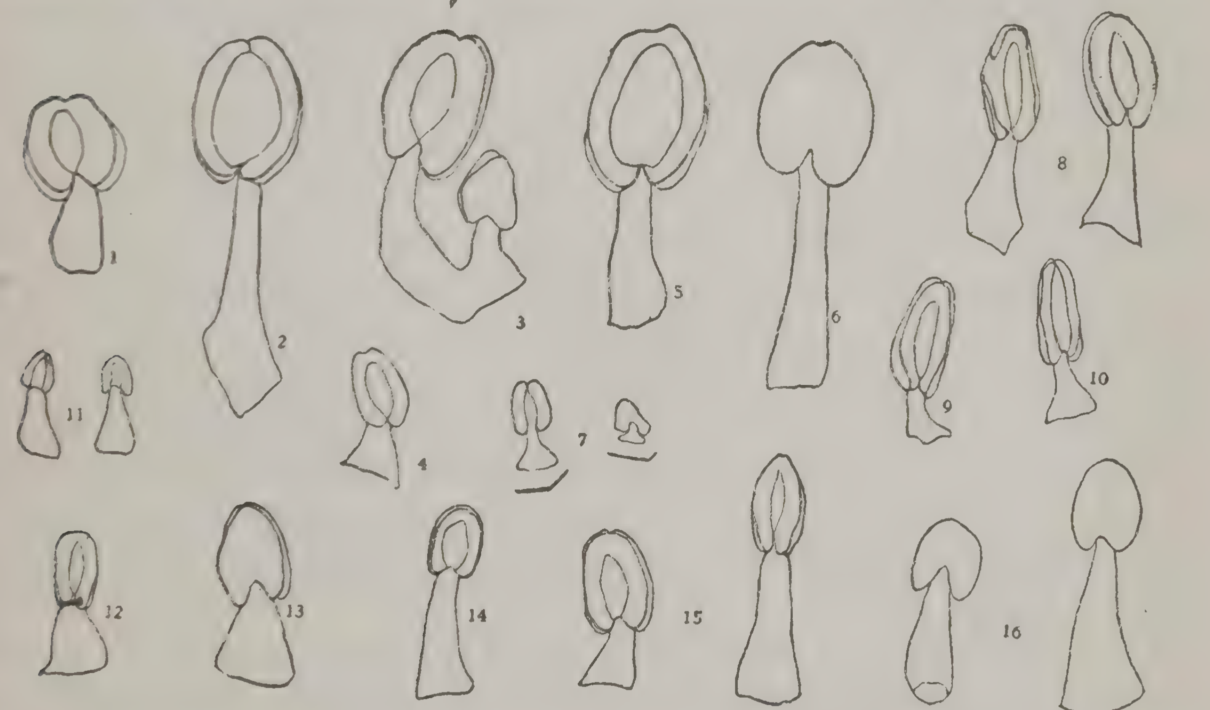

FIG. 3.—Outline camera-lucida drawings of perfect and intermediate anthers and staminodia of strawberry: *1*, *2*, and *3*, Normal anthers from tertiary flowers of a seedling of Minnesota 1017 × Progressive, Progressive, and another seedling of Minnesota 1017 × Progressive (40–1), respectively. *4*, Staminodium from a primary flower of Minnesota 1017 × Progressive (40–1). *5* and *6*, Normal anthers from Minnesota, 3 and a late primary flower of Minnesota 1017, respectively. *7*, Staminodia from a primary flower of Minnesota 1017 produced early in the spring. *8* and *9*, Intermediate anthers from primary flowers of Minnesota 3. *10*, Intermediate anther from a primary flower of Progressive. *11*, Staminodia from a pistillate flower of *F. virginiana*. *12*, *13*, and *15*, Staminodia from pistillate flowers of seedlings of Minnesota 1017 × Progressive, 2–25, 13–40, and 11–59, respectively. *14*, A staminodium from a flower of Haverland, a pistillate variety. *16*, Staminodia from a flower of Crescent, a pistillate variety which produces extremely large and prominent abortive anthers.

some of the secondary flowers throughout the season seems to be the normal condition in a number of seedlings of the cross 1017 Minnesota × Progressive (fig. 3, *3*, *4*), while among the commercial varieties there are a number which produce pistillate primary flowers early in the flowering season, while those produced later are all perfect. A few varieties which show this peculiar condition early in the spring, are Brandywine, Minnesota 3, Bederwood, Tennessee Prolific, and Staples. In figure 3, *6* represents an outline drawing of a normal anther from a primary flower of Minnesota 1017, produced late in the season, while 7 represents staminoids of the same variety taken from flowers produced early in the flowering season.

Although typically there are two rather distinct types with regard to stamen development in both cultivated and wild clones of the strawberry—namely, the perfect stamens bearing normal pollen and the staminodia of the pistillate varieties—there are apparently a series connecting these two conditions.

Figures *11*, *12*, *13*, *14*, *15*, and *16* of text figure 3 show the range of development in size of the staminodia on some pistillate plants, and *1*, *2*, *3*, *5*, and *6* show the relative size of normal anthers. In actual size the two types closely approach one another. With regard to development of reproductive tissue, there is considerable difference. The normal stamens naturally carry pollen development through to completion. The staminodia, on the other hand, never produce normal pollen, but show some variations in the extent to which development is carried. Figures A, B, C, and D, Plate 36, are photographs of cross sections of staminodia, figures A and B being cross sections of two loculi of staminodia of Crescent and Columbia, respectively, in both of which varieties the staminodia are rather prominent. Plate 36, C, is from the seedling Minnesota 1017 × Progressive, 11–59, which produces very large and prominent staminodia. Here there are distinct evidences of early degeneration of the reproductive tissue, probably pollen mother cells, although the early stages of this variety have not been studied. Figure D (Pl. 36) is from a staminodium of a pistillate variety which produces extremely small staminodia. There is no evidence of any reproductive tissue whatever having been produced.

Janczewski (*24*) has studied the stamen condition in some of the diecious species of Ribes and finds that in the pistillate flowers small stamens develop. Their internal development soon ceases and abortion of the reproductive tissue takes place. He considers that the small dark staining mass which he found in the staminodia was made up of the decomposed pollen mother cells. Often the cavity left by the breaking down of the pollen mother cells was filled with parenchyma which had grown in from the walls. Gates (*17*) found the same condition in some anthers of *Oenothera lata*. Apparently the parenchymatous tissue filling the staminodia of Crescent and Columbia is not of this origin, as early stages show no signs of the formation of pollen mother cells.

INTERMEDIATE ANTHERS.—In studying the anther types of *F. virginiana* certain clones were found which on first examination appeared to be producing normal stamens, but on closer examination were found to contain either a dark staining disintegrated mass or completely aborted microspores, the walls of which, in some cases, were disintegrating. These are apparently intermediate types between the staminodia and normal anthers. Similar types of anthers are not infrequently found in the primary flowers of many wild staminate clones.

A study of the intermediate stamens of *F. virginiana* indicates that pollen development is generally carried to the homœotypic division

or to the formation of the tetrads when degeneration occurs. This becomes apparent first through degeneration of the mother-cell wall and the cytoplasm, if the homœotypic division is taking place, leaving the spindles and chromosomes standing out sharply in this degenerate mass; or if the tetrads have already been formed, the material in which the microspores are embedded disintegrates and is followed directly by the disintegration of the microspores. Plate 36, E, shows degenerating tetrads; F shows a later stage of the same thing in which the microspores have completely degenerated; and G shows the condition found in mature anthers of this type.

Occasionally development may proceed to the formation and liberation of the microspore when, following a slight thickening of the walls, degeneration of the contents and disintegration of the microspore walls takes place. The same type of degeneration is found here as where earlier abortion takes place. The walls become thickened and, as degeneration proceeds, show a beaded appearance and finally break up into drops of a yellow oily appearing substance which makes up the mass shown in figure G (Pl. 36). In other clones of *F. virginiana* development proceeds to the liberation of the microspores from the tetrad when, following a slight development of the microspore wall, degeneration of the cell contents takes place, leaving aborted pollen of the type so characteristic of hybrids.

In the cultivated hermaphroditic varieties which produce staminodia on the early primary flowers (Pl. 36, D, G), and on some other varieties, such as Lovett, Glen Mary, and Minnesota 1017 × Progressive 9–24 (Pl. 36, B, E), these same types of anthers characterized by being small, shrunken, and bleached yellow or deep ocher are common. They show both types of degeneration—i. e., complete disintegration of the anther contents and abortion of the microspore contents following their liberation from the tetrad. Figure H, Plate 36, shows a section of a whitish yellow anther of the type shown in figure 3, *8* and *9*, from a primary flower of Minnesota 3, a variety which for the most part produces normal stamens.

Jeffrey and others have recently given emphasis to the relationship between aborted pollen and hybridity and have attempted to correlate any considerable amount of pollen abortion with a hybrid condition of the plant. Apparently in the strawberry the above type of pollen sterility and the tendency toward dieciousness are very closely related. As all degrees of stamen development may be found on a single cultivated variety of the strawberry, and on some wild plants also, from the small staminodia to well-developed stamens bearing normal pollen, it seems safe to conclude that these intermediate stamen types bearing 100 per cent aborted pollen and found in apparently pure *F. virginiana* are not the result of hybridization but are really the expression of various degrees of dieciousness.

Whether the clones of *F. virginiana* bearing these intermediate anther types are able to develop fruit, thus indicating whether they have been

derived from the pistillate forms continuing pollen development in the staminoids beyond the usual time or whether they are staminate forms in which pollen development is inhibited has not been determined experimentally. However, they appear to be of the latter type as they have been seen in flower a number of times and have shown no signs of setting fruit.

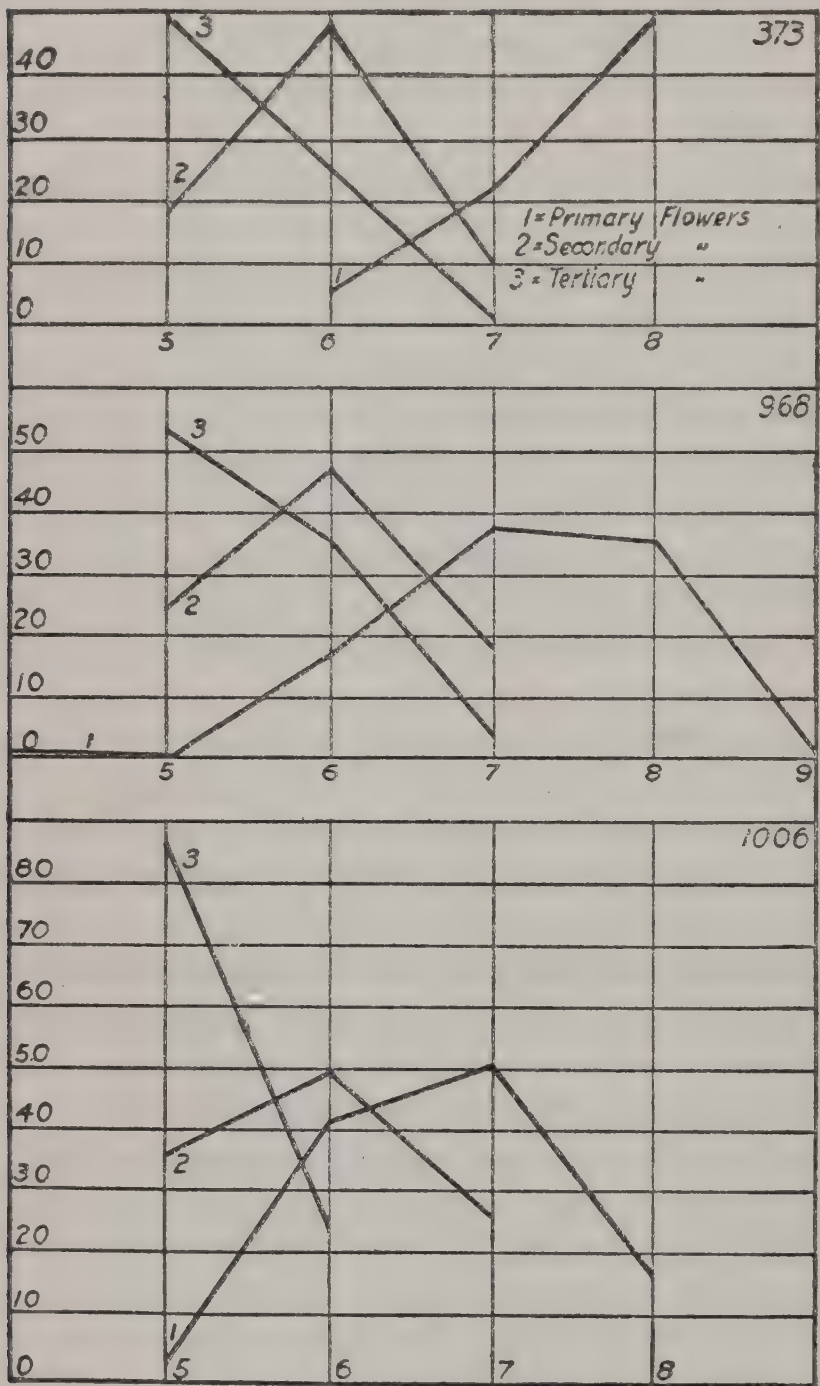

Fig. 4.—Graphs showing the relation between sepal number and flower position in the seedling varieties No. 373, 968, and 1006. The sepal number is indicated on the abscissas and the frequencies on the ordinates.

RELATION OF FLOWER PART NUMBER TO SIZE OF FRUIT

It is generally recognized by growers that toward the end of and in fact during the whole progression of the picking season of strawberries, there is a progressive decrease in the size of berries produced, but the relationship between this decrease and the position of the flowers on the inflorescence producing these smaller berries has not been so generally recognized. As has already been mentioned, strawberry flowers are typically pentamerous, but under cultivation there has been an increase in the number of parts in a portion of the flowers. This increase is most striking in the primary and secondary flowers and is only apparent to a very slight degree in the later ones. An increase in calyx-lobe number is practically always accompanied by an increase in both petal and epicalyx lobe number and as the stamens are arranged with regard to petal position there is necessarily an increase in stamen number also.

Figure 4 shows the relationship between flower position and sepal number in three seedling strawberry varieties. These show a condition typical

of our cultivated varieties. It will be seen that there is a very direct relationship between flower position and number of flower parts. In these same varieties the relationship between calyx-lobe number and size of berries was studied. The results are shown in Table I. These results indicate that there is a high correlation between flower-part number and fruit size, and as a definite relationship has been pointed out between flower position and flower-part number, it follows that the larger fruits will be developed on the early blooming primary flowers and that, as the season progresses, there will be a decrease in fruit size due to their being borne on later-blooming flowers of a higher order.

TABLE I.—*Relation of fruit size to calyx-lobe number in strawberries*

Variety.	Population.	Calyx-lobe number.			Diameter of berry.			
		Range.	Mean.	Standard deviation.	Range.	Mean.	Standard deviation.	Coefficient of correlation.
					Cm.			
Seedling 1006	274	4–8	6.350	0.925	1.4–3.8	2.326	0.343	0.482±0.031
Seedling 373	288	5–9	6.827	1.040	1.4–3.5	2.403	.532	.667± .022
Seedling 968	275	5–9	6.313	.956	1.7–3.8	2.427	.4361	.519± .03

Even a casual observation of normal large and small berries of any variety will indicate that there must be a relationship between berry size and carpel number. Table II shows the direct relationship which exists between fruit position, size of fruit and achene or carpel number in all of the fruits produced on the inflorescences studied. The fact that there is such a definite relationship between size of berry and flower position should be constantly kept in mind in the selection of breeding stock. This is apparently the point that Mr. Hubach, a southern strawberry breeder, has in mind in selecting for stalks which bear only one fruit per inflorescence (*see Darrow, 9*).

TABLE II.—*Relationship between fruit position, number of achenes, and size of fruit in the strawberry*

Variety.	Number of inflorescences studied.	Primary.			Secondary.			Tertiary.			Quaternary.		
		Number of flowers.	Average number of achenes.	Average size.	Number of flowers.	Average number of achenes.	Average size.	Number of flowers.	Average number of achenes.	Average size.	Number of flowers.	Average number of achenes.	Average size.
				Mm.			*Mm.*			*Mm.*			*Mm.*
Minnesota 3	6	7	382.28	28.0	14	224.27	17.7	21	150.9	9.8	7	92.42	7
Wildwood	7	4	229.70	23.8	17	142.7	19.0	20	88.15	11.7	3	72.7	7
F. virginiana ♀	1	1	112.00	15.0	2	116.5	13.5	4	77.0	8.3	1	70.0	6

PISTILS

MORPHOLOGY.—The carpels bearing a single orthotropous ovule are arranged in a spiral on the fleshy cone-shaped receptacle. At maturity they form dry achenes either set on the surface of the receptacle, as in *F. americana*, or in shallow or deep pits, as in *F. virginiana*. The style is inserted laterally on the inner side of the carpel and extends well above the upper portion of the ovary. The pistil number is not constant on the flowers of an inflorescence but is directly dependent upon the position of the flower.

PISTIL STERILITY AND DIECIOUSNESS.—As has been previously mentioned, *F. virginiana* is, in the wild, typically diecious, the stamens having been reduced to staminodia in the pistillate plants and the pistils, although present and apparently normal, as far as can be seen superficially, in the staminate flowers are nearly always functionless. This condition of dieciousness has apparently remained unrecognized by systematists of Fragaria. Apparently dieciousness is not confined to *F. virginiana* alone, but is typical of most of the American species, except *F. americana*, which is hermaphroditic, as is also the European species *F. vesca*.

As early as 1760 dieciousness was recognized in *F. elatior* by Duchesne (*see Fletcher, 16*), who showed that the apparent sterility of the Hautboy was in reality due to the weeding out of the unproductive male plants. He also recognized partial separation of *F. chiloensis* into male and female plants. A study of herbarium material of some of the American species of Fragaria indicates that *F. chiloensis* from Alaska to Bolivia, *F. cuneifolia* on Vancouver Island and in Washington and *F. platypetala* from the north moraine of Sir Sandford Glacier are all diecious, at least some plants produced pistillate flowers bearing staminodia, while others bore flowers with well-developed stamens and apparently normal pistils which showed no signs of setting fruit. On two plants of *F. chiloensis* from Lake Merced, California, the primary flowers of staminate inflorescences were found to have set fruit, while the remaining flowers, although well beyond the fruiting stage, showed no signs of setting. Supposedly hermaphroditic plants of *F. chiloensis* from Alaska, grown at University Farm for a number of years, produced few, if any, fruits, although they blossomed profusely.

Georgeson (*19, p. 13*), in speaking of *F. chiloensis*, which he used in his hybridization experiments, says:

> There is a decided variation among the plants; some are much more productive than others, and some appear to bear only staminate flowers, though, as a rule, the flowers are perfect.

and again (*20, p. 11*):

> The flowers are large and white and many of them staminate and sterile.

The first plants of *F. chiloensis* brought to Europe by Frezier were all pistillate and had probably been selected by him because of their fruiting propensities.

Richardson (*31, p. 176*) mentioned receiving male plants of *F. virginiana* from America. I have grown plants of *F. virginiana illinoensis* from near Dresden, Ohio, which proved to be strictly diecious. The literature on strawberry growing in this country during the early part of the last century makes many references to the necessity of growing staminate varieties for the purpose of fertilizing the pistillate forms and to the fact that these plants were unproductive of fruit, but very productive of plants and would soon dominate the garden if attention was not paid to them. Apparently they were using staminate *F. virginiana* plants as pollen producers.

A study of *F. virginiana* in various sections of Minnesota shows that for the most part this species is diecious, although some few hermaphroditic plants may be found. Of a total of 1,615 pistillate flowers of this species borne on 304 plants located in four distinct regions of the State, 1,180 set fruit, while 393 were still in the bud or blossom stage, thus indicating that practically all of the flowers of the pistillate forms are fertile if pollinated. One pistillate clone, composed of 11 plants was found, however, which produced a total of 57 flowers, 18 of which were still in the bud or flower stage; of the remaining 39 only one set seed. Another clone of this same type was found in a different region. Material of both these has been saved to test further their fertility. Plants of both of these clones, when grown under conditions more favorable to pollination, proved fertile.

In contrast to the striking fertility of most of the pistillate forms is the condition in the apparently hermaphroditic plants. Of 1,640 flowers of this type borne on 381 fruiting stalks of separate plants, 403 were in the flower or bud stage, while only 152 of the remaining 1,237 set fruit, leaving a total of 1,085 flowers which were definitely sterile; 286 plants of the 381 studied bore no fruit.

The position on the inflorescence of the flowers which set is interesting in connection with the problem of nubbins and pistil sterility in our cultivated varieties. The fact has previously been mentioned that a few flowers borne on male plants may bear only staminodia in place of stamens and that these are generally fertile. Of the 152 fruits which set, 31 were developed from this type of flower. The other flowers on these stalks were of the usual staminate form and were generally sterile, although a few instances were noted in which one of the flowers bearing normal stamens set a few achenes. Of these 31 fruits, 17 were borne on primary, 10 on secondary, 3 on tertiary, and one on a quinary flower. Five of the 152 which set were borne on flowers bearing the intermediate type of anther and of these 2 were primary and 3 secondary. The other flowers of these inflorescences bore normal anthers and were sterile. Fifteen resulted from flowers which produced some staminodia and anthers either distinctly segregated in definite portions of the flower or mixed indiscriminately; of these, 12 were from primary and 3 from

secondary flowers. The remaining 101 fruits were borne on flowers bearing a full quota of normal anthers. Of these flowers 54 were primary, 40 secondary, and 7 tertiary. While most of the fruits which set on the pistillate plants were of a regular shape, indicating a perfect or nearly perfect set of achenes, those borne on the staminates were for the most part very irregular in shape, as the achenes which set were often few in number and irregularly scattered. Often not more than one or two achenes per flower developed. Where only a few achenes developed, the typical nubbins which are so common in the latter part of the picking season in commercial plantings were produced. These results prove that *F. virginiana* is a species well on its way toward dieciousness, and, reasoning from analogy with *F. virginiana* and *F. elatior*, it may be concluded that those other American species which produce two types of plants—that is, pistillate and somatic hermaphrodites—are also diecious.

Recent investigations by Bunyard (*6*) and Fletcher (*14*) into the origin of our cultivated strawberries tend to show that they have originated from hybrids of *F. virginiana* and *F. chiloensis*, both of which are apparently diecious. If this is the case, it raises the question of the origin of our cultivated hermaphroditic forms. A study of the pistil sterility in these forms seems to indicate that they may have been derived from males which have varied in regard to pistil fertility.

Table III shows the relationship between flower position and pistil sterility in 10 hermaphroditic and 4 pistillate varieties. This table was prepared regardless of the degree of setting, whether perfect or whether the resulting fruit was a nubbin, all flowers which set any achenes being put under the heading "Set." The lower horizontal row under each of the two groups indicates the percentage of the flowers of each position which set fruit. It shows the very great increase in sterility from the first flowers to the last in both the pistillate and hermaphroditic forms, being greater in the latter than the former. This is the condition which would be expected if the cultivated hermaphrodites have been derived from males of the wild type, as the males which do set fruit in the wild exhibit a high percentage of their low fertility in the primary flowers.

The conclusion that the hermaphrodites have been derived from staminate forms rather than from pistillate is in keeping with the results found in other species, as *Lychnis* spp. (*35*) and the grape (*43*). A further study of pistil sterility was made in 15 other varieties of hermaphrodites and 3 pistillates to determine the relationship between nubbins or irregularly set fruit and flower position.

Nubbins and lack of setting of flowers have been attributed by horticulturists for the most part to lack of proper pollination or to frost injury. The first of these factors may be eliminated, however, as pollen is usually very plentiful and in a mixed planting, such as the data given in Tables III and IV were taken from, was always abundant and especially so when

the later more sterile flowers were in blossom. Further, the fact that pistillate varieties, grown in proximity to hermaphrodites, set fruit even in the early part of the season, when pollen is admittedly scarce, would argue for pollination having little to do with nubbin formation. To those who have noticed the effect of frost on strawberry flowers it will be clear that this factor may also be eliminated as a cause of irregularly set fruit, as frost, if it injures the flower at all, will blacken the entire receptacle. The possibilities of the primary flowers being "frostbitten" are much greater than the later ones, but it is the latter which generally form nubbins or are entirely sterile.

TABLE III.—*Relationship between flower position and number of fruits set in hermaphroditic and pistillate varieties of strawberries*

Variety.	Sex.	Number of stalks.	Primary.		Secondary.		Tertiary.		Quaternary.		Quinary.	
			Set.	Not set.	Set.	Not set.	Set.	Not set.	Set.	Not set.	Set.	Not set.
Reasoners 324	⚥	40	44	......	85	6	87	40	7	29	......	.....
Seedling 947	⚥	40	39	4	66	22	55	50	3	4	......	.....
Orem	⚥	40	48	......	111	11	68	45	5	7	......	.....
Lovett	⚥	40	42	......	79	3	77	28	7	13	......	.....
Seedling 893	⚥	40	42	......	72	10	58	58	4	39	......	.....
Seedling 1023	⚥	40	43	......	83	5	90	33	18	16	......	2
Abington	⚥	40	49	2	77	8	82	18	19	19	1	2
Everbearing	⚥	40	41	......	90	......	107	15	16	17	......	1
Glen Mary	⚥	40	52	......	63	19	20	44	......	8	......	.....
Seedling 924	⚥	40	52	......	74	23	41	25	2	3	......	.....
Total	......	....	452	6	800	107	685	356	81	155	1	5
Per cent	......	....	98.7	1.3	88.2	11.8	65.8	34.2	34.3	65.7	16.6	83.3
Paul Jones	♀	40	48	......	84	9	37	39	10	......	......	.....
Marie	♀	40	49	......	75	12	84	12	10	22	......	.....
July	♀	40	50	......	111	6	48	37	70	55	......	6
Wildwood	♀	8	11	......	16	2	8	1	......	3	......	.....
Total	......	....	158	......	286	29	177	89	90	80	......	6
Per cent	......	....	100	......	90.8	9.2	66.5	33.5	52.9	47.1	......	100

Table IV shows the relation between flower position, imperfectly developed fruit or nubbins, and complete pistil sterility. It corroborates what has already been pointed out, namely, that the first flowers of an inflorescence are much more fertile than the later ones. With regard to nubbins the same relationship is shown—that is, there is a gradual increase in the percentage of nubbins formed from the primary to the last flowers which open. This condition can hardly be construed as indicating anything but a morphological sterility of a portion of the pistils in those flowers which result in nubbins, if viewed with the facts in mind of the condition shown in the hermaphrodites of the wild parent species, the unquestionable sterility of many of the later flowers, and the fact that the greater percentage of these partially sterile flowers are in bloom when pollen is most abundant. If it were a question of pollination, we would expect the pistillate forms to exhibit much more sterility than the hermaphrodites, whereas they exhibit decidedly less, both with regard to the actual number of sterile flowers as well as nubbins.

TABLE IV.—*Relationship between flower position and the degree of setting in hermaphroditic and pistillate varieties of strawberries*

Variety.	Sex.	Number of stalks.	Primary.			Secondary.			Tertiary.			Quaternary.			Quinary.		
			Perfect.	Nubbins.	Not set.	Perfect.	Nubbins.	Not set.	Perfect.	Nubbins.	Not set.	Perfect.	Nubbins.	Not set.	Perfect.	Nubbins.	Not set.
Dorman	☿	20	17	2	1	53	13	2	43	23	45	8	11	55	....	....	....
Haverland [a]	☿	20	17	2	1	42	11	....	74	23	1	32	11	6	....	....	....
Parson's Beauty	☿	20	21	0	0	84	7	3	62	24	35	13	15	39	....	....	....
Bederwood	☿	20	15	5	0	36	16	....	74	28	....	28	14	5	....	....	....
Steven's Late Champion	☿	24	36	18	5	27	40	46	3	9	30	....	....	....	....	....	....
Helen Davies	☿	20	19	1	....	60	12	3	74	51	12	31	10	27	....	....	....
Senator Dunlap	☿	20	21	1	....	45	16	....	35	19	48	9	11	44	....	....	....
Pride of Delaware	☿	20	18	5	....	47	23	4	33	47	33	16	16	36	....	....	....
Minnesota 3	☿	20	22	....	....	64	2	....	70	25	6	26	16	10	....	....	....
Seedling 924	☿	20	37	....	....	73	4	....	63	2	7	1	....	....	....	....	....
Seedling 937	☿	20	30	....	....	68	4	....	95	6	9	24	8	....	....	....	....
Seedling 947	☿	20	26	1	....	39	9	....	59	4	7	11	1	14	....	....	....
Seedling 876	☿	20	27	....	....	73	....	....	85	1	2	7	1	8	....	....	....
Seedling (number lost)	☿	20	25	....	....	64	5	1	72	17	21	6	4	7	....	....	....
Seedling 778	☿	20	24	....	....	67	2	....	88	5	....	29	1	....	....	....	....
Total	....	....	355	35	7	842	164	59	930	284	256	241	119	251	....	....	....
Per cent	....	....	89.4	8.8	1.8	79.1	15.4	5.5	63.3	19.3	17.4	39.4	19.5	41.1	....	....	....
Enormous	♀	20	19	0	....	62	16	....	95	44	4	46	19	23	....	....	2
Warfield	♀	20	15	4	....	46	8	....	52	27	2	24	10	10	....	....	....
Crescent	♀	20	20	....	....	56	10	....	90	21	6	57	10	6	2	1	....
Total	....	....	54	4	....	164	34	....	237	92	12	127	39	39	2	1	2
Per cent	....	....	93.1	6.9	....	82.8	17.2	....	69.5	27	3.5	62	19	19	40	20	40

[a] Not certainly true to name.

The most characteristic type of nubbin is that in which all of the achenes set except those situated at the tip, thus producing a berry with a dead, dry tip. This raises the question as to why the tip pistil should be more sterile than those at the base.

We have already seen that when a reduction in stamen number takes place it is the youngest which are lost first, and apparently this is true also of the pistils. The pistils of the oldest flowers are decidedly more fertile than those of the later flowers, and it seems logical that the older pistils within a flower should be the more fertile. Observations on a seedling everbearing variety which in the spring produced only stamens and a small, white dome destitute of pistils in place of the ordinary receptacle, but which later in the season gradually produced normal flowers, illustrates this point. It was noticed that the first flowers which produced pistils developed only a few normal ones around the base of the receptacle, while those above gradually decreased in size until at the tip there were none. The later fruiting stalks increased the number of normal pistils until in the last fruiting stalks the entire receptacles of the first flowers were covered with normal pistils. This series of flowers, although an extreme with regard to sterility, still indicates the portion of a flower in which there is the greatest likelihood of its appearance. The question of pistil sterility should be kept in mind in the selection of breeding stock, as it is most certainly inherited in the pure species and apparently is in the cultivated varieties, as selfed seed of Glenville, a variety which rarely sets more than an occasional primary flower,

produced a number of seedlings which were as sterile as the parent. (All the progeny have not yet flowered.)

Sufficient pistillate varieties have not been studied to indicate definitely whether there is always a distinct difference between the ability of these and of the hermaphrodites to set fruit on the later flowers; however, a comparison of the percentage of fruit set on the tertiary and quarternary flowers of the pistillate and staminate varieties reported in Tables III and IV would seem to indicate that the former are decidedly more fertile than the latter, as is also the case in the wild forms. Darrow (*9*) reports that Mr. Hubach will use only pistillate varieties as the female parents because of the decrease in fruit production when hermaphrodites are used as both parents.

A cytological comparison of the sterile pistils of wild males and the sterile pistils of hermaphrodites may give further evidence as to the origin of sterility in the varieties which produce many nubbins and entirely sterile flowers. Strasburger (*39*) has shown that in a male resulting from a cross between *F. virginiana* ♀ and *F. elatior* ♂, the pistils which are apparently ready for fertilization already, in longisection, show a mass of degenerating material which contains the embryo sac mother cell. This may or may not be the condition in the pure forms of these species and in the sterile pistils of cultivated varieties.

To the commercial grower of strawberries as well as to the breeder the question of variation of fertility under varying conditions of environment or culture is of importance. Evidence which indicates that fertility is affected somewhat by environmental or seasonal conditions is given by the seedling which in the spring produced strictly male flowers but which in the summer and early autumn produced fertile pistils as well as stamens. Further, it is a matter of common observation that a bed of berries, if allowed to fruit more than one year, will produce an increasing number of small berries and nubbins. Of actual observational evidence the following indicates that growth conditions have something to do with sterility: A variety, named "Glenville" for convenience, was sent to the Station with inquiries as to why it did not set fruit. Plants of it were grown in the greenhouse during the late winter, and, although they produced numerous fruiting stalks and an average of 13 flowers per stalk, only an occasional primary or secondary flower set fruit. Some of these plants, after having been grown in benches, were put into pots and given little attention. On June 5 they were fruiting, and a count was made of the flowers which had set. On 6 fruiting stalks there was a total of 43 flowers, an average of slightly over 7 per stalk. Of these, 22 set fruit. Of 6 primaries there was 1 which set, of 12 secondaries 10, of 19 tertiaries 11, and of 6 quaternaries none set. The previous year some plants of this variety had been planted in the field; and 20 days after taking the above notes, observations were made on the field plants. Of 105 fruiting stalks examined, bearing a total of 1,292 flowers, an average of 12.3 flowers per stalk, there was a total of

20 fruits, of which 15 were borne on primary flowers, 4 on secondaries, and 1 on a tertiary. Although this variety shows an extreme case of sterility, the condition found as regards variability of sterility, may be an indication of what will be found when a thorough study is made of this point in our cultivated varieties.

Thus far the study of sterility has dealt mainly with those types of sterility induced by a decided tendency toward dieciousness in species of *Fragaria*. Another type of sterility very prevalent in cultivated varieties and undoubtedly a factor in pollination is expressed in the appearance in ripe pollen of varying amounts of defective grains. It is with this type of sterility that the remainder of this paper deals.

POLLEN DEVELOPMENT AND STERILITY

A careful cytological examination of the pollen condition in the strawberries, both wild and cultivated, was made with the objects of determining (1) the amount of viable pollen in cultivated varieties and its relation to the setting of fruit and (2) the cause of pollen abortion in plants of hybrid origin.

The material used as a basis in determining the general pollen condition in *Fragaria* spp., consisted of (a) *F. virginiana* from various parts of Minnesota, (b) *F. americana*, (c) a considerable number of cultivated varieties, and (d) seedlings under test in the course of the fruit-breeding work. The cytological study was carried on principally on the self-fertile variety Minnesota No. 3, a cross of Senator Dunlap × Pocomoke, recently introduced by the Minnesota Agricultural Experiment Station. It produces, on an average, about 50 per cent of aborted grains and so furnishes desirable material for the study of normal and abnormal pollen development. The stages in normal development were also studied in *F. virginiana*.

POLLEN CONDITION IN WILD FORMS

The recent work of Jeffry and his students on the pollen condition in wild forms puts under suspicion the genetic purity of the Rosaceae in general. The forms which have been studied most intensively, Onagraceae (*25*), *Crataegus* spp. (*37*), *Rubus* spp. (*23*), and *Rosa* spp. (*8*), show, in some species, a relatively large proportion of aborted pollen and the appearance of many subspecies, some of which appear to be hybrids. Because of this fact and with a view to comparing the pollen condition of the wild with the cultivated forms, pollen of *F. virginiana* and *F. americana* was examined.

The methods used in determining the amount of abortive pollen were as follows: Fresh flowers were collected and either were allowed to dry or were kept with their pedicels immersed in water until the anthers had dehisced. The pollen was then transferred to slides by holding the flower over a slide and giving it a few sharp taps. In this way the anthers dehisced completely onto the slide. A drop of lactic acid was then added, and a small cover slide placed over the drop, forming a

fairly permanent mount if handled carefully. The lactic acid has the advantage over water or alcohol for this purpose, as it is not volatile and seldom, if ever, breaks the pollen grains through osmotic pressure. It readily enters and expands the normal grains, while it leaves the aborted ones collapsed. It has another very distinct advantage over the more mobile liquids, in that its viscosity holds both the normal and aborted grains in place until the cover has settled firmly, while, if water is used, the empty grains have a decided tendency to wander toward the edge of the cover slide, thus invalidating the count. Where no aborted pollen was present, of course no actual counts were made; but where present, counts were made varying from 200 to 2,000, the object in each case being to include enough grains to indicate within a close range the percentage of abortion. A record was kept of the position on the inflorescence of the flower from which the pollen was collected in order to determine whether a similar relationship existed between flower position and abortion of pollen as was found to exist between flower position and stamen type in certain clones.

The results of the pollen counts on 223 flowers of *F. virginiana* are shown in Table V. They indicate that *F. virginiana* produces for the most part morphologically perfect pollen, although a few plants were found in which the percentage of aborted grains was high. If pollen condition may be taken as a criterion of species' purity it may be said that *F. virginiana* in this region is nearly a pure form. The appearance of considerable amounts of aborted pollen in a few plants might be considered as the results of the "conditions" under which these plants have been grown, but the fact that several flower types have been found in the wild may indicate that abortion is due to a condition arising from a slight degree of hybridity consequent on the intercrossing of these forms. The one primary flower which produces 100 per cent aborted pollen bore intermediate anthers and abortion was more likely due to the partial suppression of stamens, in some way connected with the tendency toward dieciousness, than to other causes which may result in sterility. This was not included in the average percentage of aborted pollen in the primary flowers for this reason.

A comparison of the pollen condition in the flowers borne on various positions shows that pollen abortion is in no way related to flower position and thus to dieciousness as are the various anther types and sterile pistils before mentioned.

In the hermaphroditic species *F. americana* nearly the same condition exists with regard to the degree of perfection of pollen as in *F. virginiana*. The result of pollen counts on 49 flowers taken from an equal number of plants are shown in Table VI and they indicate that the pollen condition of *F. americana* is normal. As it is very difficult to cross *F. virginiana* and *F. americana*, it is probable that where these two species are growing in close proximity they remain pure.

TABLE V.—*Pollen condition in wild plants of Fragaria virginiana with reference to the position of the flower on the inflorescence*

Aborted percentage.	Number of primary flowers.	Number of secondary flowers.	Number of tertiary flowers.	Number of quaternary flowers.	Number of flowers position not recorded.
0	9	11	5	....	1
1	15	18	6	....	14
2	16	5	4	....	11
3	6	5	....	....	6
4	4	3	1	....	5
5	5	2	1	....	3
6	1	3	....	....	7
7	1	4	....	....	2
8	0	2	2	....	1
9	1	2	....	....	....
10	3	1	....	....	3
11–15	4	1	3	....	6
16–20	2	1	....	1	4
21–25	2	....	1	....	2
26–30	1	....	....	....	2
31–35	1	....	....	....	1
36–40	....	....	....	1	....
100	[a] 1	....	....	....	....
Total	72	58	23	2	68
Average percentage of abortion	4.9	3.2	4.3	[b] 28.0	6.9

[a] Not included in average because borne in an intermediate type of anther.
[b] Of no significance because of few flowers.

TABLE VI.—*Pollen condition in wild plants of F. americana with reference to the position of the flower on the inflorescence*

Aborted percentage.	Number of primary flowers.	Number of secondary flowers.	Number of flowers position not recorded.
0	....	....	....
1	1	4	6
2	7	4	3
3	2	....	2
4	3	....	3
5	....	....	....
6	1	1	1
7	....	1	1
8	....	1	....
9	....	....	1
10	....	....	....
11	....	1	2
12	....	....	....
13	....	1	2
14	....	....	....
15	....	....	1
Total	14	13	22
Average percentage of abortion	2.8	4.4	5.2

Observations on the pollen condition in herbarium material of other species than those above mentioned are not conclusive with regard to the species examined because of the scarcity of material. Plants of *F. chiloensis* from Sequin, Washington, from two localities in San Francisco County, California, and from Bolivia, South America, produced perfect pollen. A single plant from Vark Hill, Cal., produced small amounts of defective grains while two plants of a clone from Lake Merced, Cal., which set fruit on the primary flowers of an otherwise staminate cluster, produced in the neighborhood of 50 per cent of aborted grains. A plant of *F. chiloensis* var. Scouleri, from Klantaak Island, Yakutat Bay, Alaska, produced perfect pollen. *F. platypetala* from Mt. Carleton, Wash., also produced perfect pollen. Although flowers of comparatively few plants of these two species have been examined, the facts seem to indicate that the pollen condition is much the same as that found in *F. virginiana* and *F. americana*.

POLLEN CONDITION IN CULTIVATED FORMS

In contrast to the nearly normal pollen condition of the wild species is the variable condition found in the cultivated forms in which practically all have a larger or smaller percentage of aborted grains. The pollen conditions in varieties, controlled seedlings of varieties, and some species-variety hybrids are given in Table VII. The percentages given are based on an average of over 600 grains per count, and indicate fairly accurately the pollen condition of the flowers studied.

TABLE VII.—*Percentage of aborted pollen in flowers, of various positions, from 120 cultivated varieties, 18 controlled seedlings of cultivated varieties, 33 selfed seedlings of one of these, 3 F_1 plants of F. chiloensis × Wilson, 7 F_1 plants of F. cuneifolia × Magoon, and 10 F_2 plants of F. vesca × F. cuneifolia. The percentages are based on an average of over 600 grains per count*

Variety.	Position of flower.			
	Primary.	Secondary.	Tertiary.	Position not recorded.
Cultivated:				
Abington	....	37.0 20.0	7.0 19.8	....
Abundance	26.5	10.5 33.0	2.0 2.8	....
Amanda	34.2	....	10.0	16.3 7.7 9.1
Arizona	63.0	70.9	....	....
Aroma	1.5	....	22.4	....
Barrymore	13.6	4.8	9.7	....
Beacon	....	1.0	....	....
Bederwood	90.8	97.0 92.0	99.3 88.7	31.7 94.3 100.0
Bradley	....	....	71.4	....
Brandywine	21.0	....	14.1	12.5 5.9
Brown Beauty	....	10.5	9.7	....
Charles	....	39.0	36.7	....
Chesapeake	....	47.0	....	....

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Variety.	Position of flower.			
	Primary.	Second-ary.	Tertiary.	Position not recorded.
Cultivated—Continued:				
Clara	...	...	1.8	...
Climax	...	42.5	24.8	...
Clyde	45.5	...	61.4	38.2
Commonwealth	...	2.0	...	...
Cooper	13.0	...	32.3	...
Corsican	...	27.0	9.4	10.5
Darlington	...	56.0	11.2	...
Deacon	...	1.8	...	...
Dewdrop	38.5	...	...	...
Dorman	...	...	7.7	10.3 39.6 38.5
Duncan	...	2.0	16.6	...
Early Giant	23.2	...	35.6	...
Early Jersey Giant	38.0	...	6.0	...
Early Ozark	50.0	...	42.3	...
Ekey	1.5	0.0	10.6	...
	0.0	...	...	...
Enhance	7.9	...	37.4	...
Everbearing	...	79.6	...	...
Ewell's Early	...	61.5	10.8	...
Excelsior	66.0	...	1.3	...
Fendell	...	12.0	...	...
First Quality	54.0	...	68.4	...
Frances E. Willard	4.9	...	34.2	...
Fitting Eclipse	...	...	5.2	...
Gandy	66.8	87.4	61.0	...
		59.1	...	...
Gill	68.8	...	48.0	...
Glen Mary	63.7	...	46.7	51.5 44.9 100,0 97.3 100.0 98.0
Glenville	...	...	...	17.6
Gold Dollar	63.1	...	...	...
Gold	...	...	50.0	...
Good Luck	56.0	31.0	...	...
Goree	53.0	...	44.0	...
Grand Marie	1.0	...	42.9	...
Hanbeck Beauty	69.9	...	42.9	...
Haverland[a]	27.1	...	36.5	...
Ideal	63.0	...	56.0	3.0
	39.7	...	43.5	67.8
Indiana	20.5	...	15.8	...
Jas. Todd	82.3	...	69.6	...
Jerome	8.6	...	...	51.0 14.3
Jessie	6.5	...	50.0	...
Jewell Improved	9.5	...	...	...
Jewell	...	...	38.5	...
Jocunda	39.5	...	72.4	...
Joe	60.0	...	33.1	...
Kevitt Wonder	80.8	...	...	...
King Edward	33.8	...	12.6	...
Klondike	73.7	...	50.1	...
	54.0	84.3	...	53.7
Late Jersey Giant	6.8	...	13.0	11.8
	2.7	5.6	6.8	15.0
Lea	86.6	70.2	...	...
	44.9	46.5	...	...
Longfellow	97.7	...	54.7	...
	46.0	19.1	43.5	...
Lovett	99.0	...	...	100.0
	84.3	...	...	100.0
	76.9	83.9	86.9	95.0 92.5 85.8 84.0

[a] Not certainly true to name.

TABLE VII.—*Percentage of aborted pollen in flowers, of various positions, from 120 cultivated varieties, 18 controlled seedlings of cultivated varieties, 33 selfed seedlings of one of these, 3 F_1 plants of F. chiloensis × Wilson, 7 F_1 plants of F. cuneifolia × Magoon, and 10 F_2 plants of F. vesca × F. cuneifolia*—Continued

Variety.	Position of flower.			
	Primary.	Secondary.	Tertiary.	Position not recorded.
Cultivated—Continued:				
Magoon		31.9		
Malinda	11.3		8.6	
Manhattan	32.6 44.5 44.1		50.8	
Marshall	6.0			24.8 15.5 33.3 25.0 25.0
Mascot	82.0		43.5	
Michell Early	65.6		21.7	
Miller	97.5		18.2	
Minnesota 3	28.7 41.9 47.5	52.1 60.4 14.2	47.1 41.8 41.9 39.5	
Missionary	86.8 45.3	38.0	57.5 81.4	
Model	1.5		4.0	
New Home	47.4		67.2 50.5	
New York	3.5		1.0	
Nick Ohmer			15.0	38.4
Ohio Boy	17.2		45.5	81.1 93.0 73.0
Orem	94.2		25.0	
Oswego	10.2		19.8	
Palmer	96.3		72.5	
Panama			11.5	
Pan American	21.7		10.3	
Park Beauty			29.6	
Parson's Beauty			22.0	60.0
Pearl			53.8 43.5	
Pennell	44.4		39.9	50.0 56.1
Pineapple	20.0		16.2	9.0 18.0 16.9 15.4
Pitcher Eclipse				49.8
Progressive	75.3		41.8	63.8 28.1 14.5
Prolific	26.7		42.9 7.8	
Pride of Delaware				62.0
Providence	24.0	26.4	22.3	100.0
Purcell Early				100.0
Purcell			55.7	
Reasoner 324			53.5	
Rewatisco			91.5	35.6 37.4
Ridgeway			23.1	40.0 45.4 82.6
Sample	29.6		19.2	44.3 31.3 60.5 29.6
Saratoga			61.7	
Saunders			46.7	
Seedling 373	62.0		30.7	84.4 85.9
Seedling 585	7.0		0.5	
Seedling 702	85.1		24.1	
Seedling 753	54.0		3.0	
Seedling 776	12.5	3.5	3.9 6.0	

TABLE VII.—*Percentage of aborted pollen in flowers, of various positions, from 120 cultivated varieties, 18 controlled seedlings of cultivated varieties, 33 selfed seedlings of one of these, 3 F_1 plants of F. chiloenis × Wilson, 7 F_1 plants of F. cuneifolia × Magoon, and 10 F_2 plants of F. vesca × F. cuneifolia*—Continued

Variety.	Position of flower.			
	Primary.	Secondary.	Tertiary.	Position not recorded.
Cultivated—Continued:				
Seedling 778	24.8		20.0	25.5 14.2 9.9
Seedling 813			77.5	58.0
Seedling 825	79.8		54.1	
Seedling 893			61.8	65.0
Seedling 908			29.6	17.5 5.0 11.3
Seedling 923	9.0 13.0		15.2	
Seedling 924			31.0	
Seedling 927			10.0	
Seedling 947			59.3	96.0 55.4
Seedling 1010			14.3	
Seedling 1017			2.2	20.5 24.5 2.7 2.0
Seedling 1043				8.9
Seedling 1045			13.8	
Senator Dunlap			45.9	36.6 20.0 29.4
Son's Prolific	18.5	25.4		
South Dakota		15.9	20.0 34.9	36.7 50.9
Splendid				58.4
Splendid × Dunlap	100.0	99.9	99.9 98.8	100.0
Staples			46.3 28.6	37.5 51.9 51.0
Steven's Late Champion				24.8 63.6 70.6 50.6
St. Louis			57.9	
Success			1.0	
Sweetheart	52.5	55.4	52.1 69.0	
Tennessee Prolific			75.8	
Texas		25.0	40.0 45.2	
Three Ws			4.6	
Twilley			2.2	
Uncle Jim			10.2	
Warren			80.6	
Warfield [a]			9.5	
Wm. Belt			1.6	
Wilson		43.3		
Winner			1.2	
Wolverton			55.4	
Wonder			7.8	
Average	43.2	38.3	34.2	45.9
Selfed seedlings of Seedling 778:				
24-3		47.1		
24-4	77.2	52.4		
25-1		27.9		
25-2	77.0		17.0	
25-3	17.0	6.0		
25-6	49.2			
26-1		9.5		
26-4				43.0
27-5	52.3		52.2	
28-3	24.5			
53-4	23.5			
54-2	21.1			

[a] Not certainly true to name.

TABLE VII.—*Percentage of aborted pollen in flowers, of various positions, from 120 cultivated varieties, 18 controlled seedlings of cultivated varieties, 33 selfed seedlings of one of these, 3 F_1 plants of F. chiloensis × Wilson, 7 F_1 plants of F. cuneifolia × Magoon, and 10 F_2 plants of F. vesca × F. cuneifolia*—Continued

Variety.	Position of flower.			
	Primary.	Secondary.	Tertiary.	Position not recorded.
Selfed seedlings of Seedling 778—Continued:				
56-4			25.3	
65-2		9.9		
65-3		22.5 19.4		
65-4		52.4 42.5		
66-1				32.0
66-2	94.2	99.0		
66-3		43.9		
67-1	25.8	38.1		
67-3			41.3	
67-4	99.0			
67-5	9.1	10.6 22.2		
71-5		21.3		
71-7	46.0			
72-1	100.0	33.1		
72-2			25.9	
72-3	0.4			
72-4	49.1	50.8	34.9	
72-6		52.7		
73-3			1.8	
73-4			62.8	
73-5	34.7	61.0		
Average	47.1	36.1	32.7	37.5
3 F_1 plants of *F. chiloensis* × Wilson:				
1			95.0	
2			77.1	
3		58.0		
Average				76.7
7 F_1 plants of *F. cuneifolia* × Magoon:				
1			5.2	
2	15.0			
3	2.0			
4	21.4			
5		18.2		
6	16.4			
7		29.1		
Average				15.3
10 F_2 plants of *F. vesca* × *F. cuneifolia:*				
1	11.2			
2	7.7			
3	8.3			
4	8.0			
5		19.3		
6	7.3			
7		7.0		
8	11.9			
9		5.2		
10			3.1	
Average				8.9

The most striking fact exhibited by the counts in Table VII, other than the general presence of some aborted pollen, is the variability of the pollen condition within a variety. Abington, for instance, shows a range of from 7 to 37 per cent of abortive pollen. Abundance from 2 to 33 per cent, and Bederwood, which usually produces a high per-

centage, in one instance produced as low as 31.7 per cent of abortive grains. Numerous other equally striking variations in pollen abortion will be evident by referring to the table.

In view of the variability shown above, the pollen condition was determined in all of the flowers from two inflorescences of Minnesota 3 to determine what variations occur in flowers grown under so nearly similar conditions. The results of these counts are presented in Table VIII and exhibit as great variability within the flowers of a cluster as is found between flowers from separate plants. In one stalk the range is from 31.2 to 91.1 per cent, in the other it is from 21.4 to 40.3 per cent, while the range in other counts of the same variety (Table VII) from different plants is from 14.2 to 60.4 per cent.

TABLE VIII.—*Percentage of aborted pollen in all of the flowers from two stalks of Minnesota 3*

Stalk.	Primary.	Secondary.	Tertiary.	Quaternary.
Stalk I	40.3	29.9	21.4	....
		30.6	....	....
Stalk II	(a)	91.1	42.3	42.6
		63.5	68.2	42.7
		70.7	31.2	....
		78.9	49.7	....

a Anthers intermediate.

In view of the variability shown between different plants of the same variety and between different flowers of a single inflorescence, a study was made of the variation in pollen conditions of individual anthers within a single flower. The pollen of nine anthers, from a flower of Seedling 778 was studied, four of which were from the outer parapetalous series, three from the middle antipetalous series, and two from the inner long filamented antisepalous series. The results, given in Table IX, show a greater range of variability between the anthers of a single flower than was exhibited by the five separate flowers of the same variety reported in Table VII. Because of the extremely great variability in pollen condition shown by some varieties, as Abington, Abundance, Aroma, Darlington and others, which often produce nearly perfect pollen (as far as can be determined by this method of study), while at other times, apparently under the same conditions, high percentages of abortive pollen are produced, too much stress should not be laid on a few scattered observations in determining whether a species is pure or of hybrid origin until more is known of the factors which produce such great variability. It may, of course, be argued, and logically, that a large number of the grains in the anthers, which produce nearly perfect pollen, are truly abortive, but have developed beyond the stage where

degeneration can be discovered by a superficial examination. The possibility of this being the case will be shown later. Nevertheless the factors causing a relatively high degree of pollen abortion in apparently pure species have been so little studied that to assign hybridity as the only cause is, to say the least, presumptive.

TABLE IX.—*Variation in percentage of pollen abortion in anthers from one flower of Seedling 778*

Stamen position.		
Parapetalous.	Antipetalous.	Antisepalous.
29.18	33.65	27.27
50.00	38.65	41.87
41.70	17.09	....
34.61	....	....

POLLEN GERMINATION TESTS

In view of the possibility of many of the apparently normal grains being in reality abortive, attempts were made to determine the exact amount of fertile pollen without regard to its apparent morphological condition. The usual method of pollen germination in Van Tieghem cells was employed. The results were disappointing, so far as a determination of actual condition of individual grains was concerned; but nevertheless some suggestive conclusions may be drawn from them. In all, 450 tests were made, comprising 28 of *F. americana*, 45 of *F. virginiana*, and 377 of cultivated varieties. The results obtained under carefully controlled conditions were very erratic. Solutions of cane sugar in distilled water were used in concentrations varying from 7 to 60 per cent. Pollen germinated to a very slight degree at both of these extremes, but the optimum concentration ranged between 35 and 45 per cent.

Temperature is an important factor in pollen germination, since at ordinary room temperature there was practically no germination, while if the cultures were placed in a warmer portion of the room, at a temperature of about 90° F. sometimes a high germination resulted. Other tests, carried on under as nearly identical conditions as possible, often gave entirely negative results. Tests made in an electric oven, at 95° F., showed at times a high percentage of germination, while at others the germination was very low. Although the proper conditions for germination could not always be produced, yet enough evidence was obtained to conclude that wherever any normal pollen is present, a portion of it is likely to have the power of germination, for in several cases where over 95 per cent of the pollen was abortive some of the morphologically perfect grains produced normal tubes.

BAGGING TESTS

The final test for the fertility of pollen is its behavior when used in pollination. The simplest method of testing pollen fertility is to bag the flowers before they open. This method has been used very extensively by several investigators in self-sterility studies of the pear, apple, cherry, peach, and grape and has been found to be efficient under favorable conditions. Observations by growers upon large blocks of any of the hermaphroditic varieties of strawberries agree that strawberries are all self-fertile, physiologically, wherever normal pollen is produced, so that the question of self-sterility does not enter into the problem.

Bagging tests were made on 106 varieties and 40 unnamed seedlings produced at the Minnesota Fruit Breeding Station. The detailed results and summary of these tests are given in Table X, and show (1) that in the hermaphroditic varieties studied no physiological self-sterility exists; (2) that wherever morphologically normal pollen is present fertilization takes place; and (3) that the extent of fertilization is dependent upon the percentage of normal pollen produced.

TABLE X.—*Degree of setting of the fruits of various positions on the inflorescence on 98 hermaphroditic varieties and 39 seedlings of strawberries when bagged*

Variety.	Number of stalks bagged.	Primary.			Secondary.			Tertiary.			Quaternary.			Number of flowers.	Total.		
		Perfect.	Nubbins.	Not set.	Perfect.	Nubbins.	Not set.	Perfect.	Nubbins.	Not set.	Perfect.	Nubbins.	Not set.		Perfect.	Nubbins.	Not set.
Abington	15	2	....	....	8	2	1	....	6	9	....	1	6	35	10	9	16
Abundance	5	....	3	....	2	5	3	2	5	15	....	3	10	48	4	16	28
Amanda	3	....	1	....	....	5	1	6	3	4	2	3	5	30	8	12	10
Arizona	2	....	1	1	....	4	....	2	3	1	....	....	3	15	2	8	5
Aroma	4	....	3	....	5	3	....	8	1	3	....	....	....	23	13	7	3
Barrymore	2	1	....	....	2	....	2	1	....	5	....	....	....	11	4	....	7
Bederwood	4	....	3	1	....	8	2	5	14	....	3	1	....	37	8	26	3
Bradley	5	....	1	2	3	9	5	7	3	8	....	1	11	50	10	14	26
Brandywine	3	....	2	....	10	....	....	8	4	5		4	....	36	22	6	8
Brown Beauty	3	....	2	1	....	3	5	6	4	7	....	2	7	3	6	11	20
Charles I	4	1	2	....	2	5	3	1	6	13	....	....	....	37	4	13	16
Chesapeake	4	2	2	....	6	5	....	8	4	....	....	4	2	33	16	15	2
Clara	1	....	1	....	2	1	....	3	....	1	....	....	2	33	5	2	3
Climax	4	2	1	1	12	....	....	14	1	2	....	2	2	10	28	4	5
Clyde	2	2	....	....	....	4	3	....	4	9	....	....	8	37	2	8	20
Cooper	1	1	....	....	1	3	....	....	....	....	....	....	....	30	2	3	....
Commonwealth	2	2	....	....	5	....	....	6	....	....	2	....	....	5	15	....	....
Corsican	4	3	2	....	1	4	7	3	4	15	....	....	3	15	7	10	25
Darlington	2	3	1	....	4	2	....	4	....	4	....	....	1	42	11	3	5
Dorman	3	....	5	....	3	5	2	3	2	1	....	1	4	19	6	13	7
Duncan	1	....	1	....	....	1	1	2	....	....	....	1	1	26	2	3	2
Early Jersey Giant	2	....	....	....	2	2	1	4	2	....	....	....	....	7	6	4	1
Early Ozark	2	2	....	....	3	1	1	1	....	3	....	....	....	11	6	1	4
Ekey	6	....	6	2	4	5	8	....	3	16	2	3	9	11	6	17	35
Enhance	2	1	1	....	1	2	2	....	3	3	....	1	2	58	2	7	7
Ewell Early	2	1	1	1	3	2	1	3	1	2	2	....	10	16	9	4	14
Excelsior	4	7	1	....	8	3	....	....	....	6	....	....	....	27	15	4	6
First Quality	6	2	2	1	9	5	....	12	6	2	5	....	3	25	28	13	6
Gandy	2	....	1	....	....	7	....	3	2	3	....	....	....	47	3	10	3
Gill	4	....	1	....	3	10	....	1	5	....	....	....	2	16	4	16	2
Glen Mary	1	1	....	....	4	....	1	1	....	1	....	....	....	22	6	....	2
Gold Dollar	5	4	2	1	9	4	2	12	1	....	1	1	5	8	26	8	8
Good Luck	2	....	2	....	....	7	....	4	4	1	....	2	4	42	4	15	5
Goree	1	....	1	....	1	1	1	1	....	11	....	....	....	24	2	2	12
Grand Marie	4	2	3	....	3	1	4	4	3	3	1	1	7	16	10	8	14
Hanbeck Beauty	3	....	4	1	....	7	3	....	3	7	....	....	....	32	....	14	11
Haverland [a]	2	....	....	....	4	....	....	2	....	....	....	....	2	25	8	6	....

[a] Not certainly true to name.

TABLE X.—*Degree of setting of the fruits of various positions on the inflorescence on 98 hermaphroditic varieties and 39 seedlings of strawberries when bagged*—Continued

Variety.	Number of stalks bagged.	Primary.			Secondary.			Tertiary.			Quaternary.			Number of flowers.	Total.		
		Perfect.	Nubbins.	Not set.	Perfect.	Nubbins.	Not set.	Perfect.	Nubbins.	Not set.	Perfect.	Nubbins.	Not set.		Perfect.	Nubbins.	Not set.
Ideal	7	4	3	2	7	7	11	2	4	18	....	....	6	64	13	14	37
Indiana	2	....	1	....	....	5	....	....	7	2	....	3	....	18	....	16	2
Jas. Todd	4	1	2	....	....	10	....	....	7	7	....	....	1	28	1	19	8
Jerome	4	....	1	1	3	3	5	....	3	15	....	....	19	50	3	7	40
Jessie	3	3	2	....	3	6	1	1	4	....	....	....	....	20	7	12	1
Jewell Imp.	4	....	1	2	4	3	3	5	5	6	1	2	11	43	10	11	22
Joe	3	....	4	....	5	2	1	4	3	....	....	....	2	21	9	9	3
Kevitt Wonder	2	1	1	....	4	....	....	....	4	2	....	....	5	17	5	5	7
King Edward	4	1	4	1	5	4	7	4	2	10	....	....	....	38	10	10	18
Klondike	3	....	1	....	5	5	....	....	10	....	....	....	1	22	5	16	1
Late Jersey King	4	1	2	....	4	5	....	10	3	4	2	1	....	32	17	11	4
Lea	4	1	3	....	....	10	....	2	7	4	....	3	6	36	3	23	10
Longfellow	3	....	2	1	....	3	2	....	....	11	....	....	1	20	....	5	15
Lovett	4	....	2	2	....	8	1	1	11	2	2	2	2	33	3	23	7
Manhattan	8	....	6	1	2	19	2	6	28	3	....	....	3	70	8	53	9
Marshall	2	....	2	....	....	5	....	1	3	2	....	....	....	13	1	10	2
Mascott	4	....	5	....	....	14	....	2	7	7	....	....	1	36	2	26	8
Michell Early	1	....	....	....	....	1	1	....	7	....	....	4	1	14	....	12	2
Miller	3	....	2	....	....	10	1	4	11	5	....	4	8	45	4	27	14
Missionary	5	2	3	....	1	8	....	5	11	1	....	7	5	43	8	29	6
Model	2	2	1	....	....	3	....	5	6	....	3	....	....	20	10	10	....
Molinda	2	1	1	....	....	3	....	2	1	2	....	....	....	10	3	5	2
New Home	4	....	5	1	4	7	1	....	4	18	....	....	5	45	4	16	25
New York	4	....	5	....	....	1	9	1	3	8	....	....	....	27	1	9	17
Nich Ohmer	1	....	....	....	2	....	....	....	2	....	....	....	3	7	2	2	3
Ohio Boy	6	....	4	1	....	19	2	1	22	10	1	4	6	70	2	49	19
Orem	3	....	3	....	....	10	....	....	9	8	....	....	....	30	....	22	8
Oswego	6	3	1	....	....	13	3	....	6	21	....	1	1	49	3	21	25
Palmer	3	....	2	1	....	3	5	....	4	7	....	5	2	29	....	14	15
Pan American	6	1	3	1	1	5	7	....	12	9	....	6	20	65	2	26	37
Parson Beauty	4	....	6	....	....	6	11	....	8	13	....	....	3	47	....	20	27
Pearl	4	....	6	....	3	9	....	4	13	6	....	....	6	47	7	28	12
Pennell	2	1	....	....	5	....	....	....	4	2	....	....	7	19	6	4	9
Pineapple	4	....	5	....	2	9	2	....	6	5	....	....	1	30	2	20	8
Pitcher Eclipse	3	....	2	....	2	4	1	1	12	....	....	....	....	22	3	18	1
Pride of Delaware	4	1	4	....	5	8	....	5	6	10	....	1	6	46	11	19	16
Pride of Minnesota	6	2	6	....	2	12	1	....	14	7	....	9	9	62	4	41	17
Prolific	6	3	2	....	9	2	3	8	5	12	....	....	2	46	20	9	17
Providence	4	2	2	....	4	7	....	5	1	9	....	....	3	33	11	10	12
Reasoner 324	2	....	4	....	....	7	....	2	10	....	....	5	....	28	2	26	....
Rewatisco	3	1	2	....	5	4	....	4	6	1	....	....	....	23	10	12	1
Ridgway	3	....	3	....	4	7	....	4	6	5	....	....	....	29	8	16	5
Sample	3	2	1	....	1	4	2	....	11	3	....	....	12	36	3	16	17
Saratoga	2	....	2	....	1	5	....	....	5	8	....	....	3	24	1	12	11
Saunders	2	....	2	....	....	8	....	2	4	7	....	....	1	24	2	14	8
Senator Dunlap	2	2	....	....	2	1	1	2	....	4	....	....	4	16	6	1	9
Son's Prolific	4	....	3	1	5	6	....	6	4	6	1	....	11	43	12	13	18
South Dakota	3	2	2	....	3	4	....	3	2	8	....	1	7	32	8	9	15
Staples	4	3	2	1	5	4	2	6	1	11	1	....	5	41	15	7	19
Steven's Late Champion	5	....	4	....	3	10	1	4	9	15	....	1	6	53	7	24	22
St. Louis	3	1	2	....	....	8	....	....	2	4	....	....	....	17	1	12	4
Success	2	2	....	....	1	1	....	1	3	....	....	....	1	9	4	4	1
Tennessee Prolific	2	....	3	1	....	6	....	....	4	....	....	....	....	14	13	....	1
Three Ws	1	....	....	....	....	1	2	....	1	5	....	....	1	10	2	....	8
Twilley	1	....	2	....	....	5	1	....	1	2	....	....	....	11	....	8	3
Uncle Jim	2	1	2	1	....	4	4	....	1	5	....	....	5	23	1	7	15
Warfield	3	1	1	1	4	7	....	6	3	4	2	3	6	38	13	14	11
Warren	1	1	1	....	3	1	1	....	1	2	....	....	....	10	4	3	3
Wm. Belt	4	6	2	....	7	5	1	4	....	....	5	....	3	33	22	7	4
Winner	2	....	1	2	....	5	1	....	3	5	....	2	1	20	....	11	9
Wolverton	2	3	....	1	5	2	....	....	5	5	....	....	....	21	8	7	6
Minnesota 3	4	1	3	....	....	10	....	....	8	3	1	1	2	29	2	22	5
Seedling 14	2	....	1	2	....	2	4	....	....	8	....	....	7	24	....	3	21
Seedling 114	[a] 1	....	1	....	....	6	....	....	8	2	2	5	7	31	2	20	9
Seedling 130	[a] 3	....	4	....	1	4	5	3	2	6	1	2	4	32	5	12	15
Seedling 97	[a] 2	1	1	1	....	7	....	1	4	5	2	2	2	26	4	14	8
Seedling 15	1	....	....	....	....	1	3	....	1	4	....	....	2	11	....	2	9
Seedling 123	[a] 1	....	....	....	....	....	3	....	5	1	....	....	1	10	....	5	5
Seedling 123	[b] 1	....	....	....	....	....	....	....	....	....	....	....	....	11	....	....	....
Seedling 89	2	....	2	1	....	3	6	6	3	9	3	....	....	33	9	8	16

[a] One bag split or tip open, allowing the possibility of cross-pollination.
[b] Of a total of 11 flowers, none set. The pollen of this variety has not been examined.

TABLE X.—*Degree of setting of the fruits of various positions on the inflorescence on 86 hermaphroditic varieties and 39 seedlings of strawberries when bagged*—Continued

Variety.	Number of stalks bagged.	Primary.			Secondary.			Tertiary.			Quaternary.			Number of flowers.	Total.		
		Perfect.	Nubbins.	Not set.	Perfect.	Nubbins.	Not set.	Perfect.	Nubbins.	Not set.	Perfect.	Nubbins.	Not set.		Perfect.	Nubbins.	Not set.
Seedling 40	2	....	1	....	2	2	7	4	3	10	....	....	3	32	6	6	20
Seedling 168	2	1	....	3	....	2	6	....	....	4	....	1	5	22	1	3	18
Seedling 585	2	....	1	1	....	2	3	4	3	1	4	1	4	24	8	7	9
Seedling 753	[a] 2	....	3	....	....	4	1	4	3	2	1	....	4	22	5	10	7
Seedling 876	[a] 2	1	....	....	....	6	....	4	5	....	....	1	4	21	5	12	4
Seedling 703	1	....	1	....	....	3	....	1	1	4	....	....	4	14	1	5	8
Seedling 778	[a] 2	....	1	....	....	3	2	2	6	1	8	....	1	24	10	10	4
Seedling 937	2	....	4	....	....	5	2	1	8	3	....	5	2	30	1	22	7
Seedling 1065	2	1	2	....	3	3	....	3	5	2	2	....	3	24	9	10	5
Seedling 1009	2	....	1	....	....	7	....	2	1	3	1	1	2	18	3	10	5
Seedling 845	2	....	1	1	1	2	2	1	2	4	1	1	....	16	3	6	7
Seedling 901	1	....	1	....	....	2	....	....	1	1	....	....	....	5	....	4	1
Seedling 1018	2	....	2	....	....	2	3	....	3	6	....	....	....	16	....	7	9
Seedling 1017	2	....	2	....	2	2	....	6	1	....	....	....	....	13	8	5	....
Seedling 585	2	1	1	....	....	6	3	....	....	11	....	....	....	22	1	7	14
Seedling 702	2	....	2	....	....	3	1	1	2	7	....	1	4	21	1	8	12
Seedling 753	2	....	....	....	....	5	....	1	8	....	....	....	....	14	1	13	....
Seedling 778	4	3	1	....	5	2	....	7	2	....	....	2	1	23	15	7	1
Seedling 776	7	5	3	....	6	10	....	1	15	4	....	....	....	44	12	28	4
Seedling 908	2	1	3	....	4	2	3	4	....	2	3	....	....	22	12	5	5
Seedling 923	4	....	3	....	....	8	....	6	3	2	....	....	....	22	6	14	2
Seedling 924	1	1	....	....	....	3	3	....	....	6	....	....	....	13	1	3	9
Seedling 937	1	....	1	....	....	3	....	....	4	....	....	....	3	11	....	8	3
Seedling 947	3	....	3	....	1	6	2	....	8	....	....	....	1	21	1	17	3
Seedling 1010	5	....	5	2	1	7	11	....	7	15	1	....	8	57	2	19	36
Seedling 1017	3	....	4	....	....	4	5	4	7	....	....	....	....	24	4	15	5
Seedling 1023	3	3	....	1	8	1	....	10	....	2	....	....	....	25	21	1	3
Seedling 1026	2	....	....	1	....	4	....	1	2	2	....	....	6	16	1	6	9
Seedling 1045	1	....	1	....	....	2	....	....	2	2	....	....	2	9	....	5	4
Splendid×Dunlap	2	....	1	1	....	....	4	....	....	8	....	....	4	18	....	1	17
Total	....	113	260	48	285	624	234	337	587	646	70	114	410	....	....	....	....
Per cent	....	26.8	61.8	11.4	24.9	54.6	20.5	21.5	37.4	41.1	11.8	19.2	69	....	....	....	....

[a] Two bags split or tip open, allowing the possibility of cross-pollination.

Pollination in unbagged flowers is for the most part dependent upon bees and small insects and upon the anthers becoming erect and partially folding about the receptacle while dehiscing, thus dropping the pollen on the stigmatic surfaces. In the bagged flowers the insects are eliminated, and thus the most efficient natural means of pollination is lost. As a consequence many of the bagged varieties produced many nubbins, but no case of complete self-sterility was found where morphologically perfect pollen was present.

If the percentages of total perfect fruits, nubbins, and sterile flowers of each flower position in Table IV are compared with those in Table X, it will be seen that (1) the setting under bags is very much poorer than in the open, both with regard to the number of perfect fruits set and also with regard to the actual number of flowers which set any achenes, and (2) the primary flowers are decidedly more fertile than the later ones, as, even under the adverse conditions of pollination within the bags only 11.0 per cent of the primary flowers were sterile, while 20 per cent of the secondary. 40.7 per cent of the tertiary, and 68.5 per cent of the quaternary flowers were sterile, in spite of the very great increase in amount of

pollen present in the bags while the later flowers were in condition for pollination.

In order to determine to what extent parthenogenesis or parthenocarpy might possibly enter into the above results, bags were put over 67 clusters of 22 pistillate varieties. Of a total of 661 flowers covered, 55 set some achenes. Of these, 52 were found in 6 bags which had been accidentally split, thus accounting for the probable pollination by insects. Of the remaining three fruits, which developed in apparently tight bags, two bore 1 achene each and the other 7. Significance can hardly be attributed to the setting of these few achenes, since the chances for accidental pollination, to this extent, are relatively great. It may therefore be coucluded that parthenogenesis does not exist in the cultivated strawberry.

A condition which might possibly be attributed to parthenocarpy occurred in the Buster variety, in which 9 flowers of the 22 bagged showed a very decided development of the achenes with no accompanying development of the receptacle. These achenes contained no embryos. In the strict sense of the word parthenocarpy in the strawberry could only be applied to a development of this kind. A more comprehensive use of the term might include the development of the fleshy receptacle. Ordinarily, flowers which set only one or a few achenes develop the fleshy receptacle only at corresponding points, due probably to the stimulus of fertilization. In perfect varieties receptacles are often found in which development has taken place not only at the base of the pistils but also about the base of the stamens. In 3 out of 10 bagged flowers of the pistillate Red Bird variety, the fleshy receptacle developed about the base of the staminodia forming a red fleshy circle about the dried pistils. Two flowers of Crescent, also an imperfect variety, developed normal fleshy berries, one bearing one achene and the other none. Apparently these receptacles developed without the stimulus of fertilization in the same way as that at the base of the staminodia in Red Bird.

POLLEN DEVELOPMENT

As Mendelian and, in fact, most genetic results are dependent upon the segregation of determiners during the formation of gametes and to their recombination again at the time of fertilization, any processes which interfere with the normal procedure should be carefully studied and, if possible, their nature determined.

There are a number of ways in which the normal order may be disturbed, at least there are various outward expressions of them. The condition in the *Phylloxera* spp., as pointed out by Morgan, in which half of the spermatids degenerate regularly, while the other half continue and form normal spermatozoa, and the relationship between degeneration and the absence of the accessory chromosome is so well known that it needs no comment. Gates (*18*) has shown that in

Oenothera lata the early abortion of the male generative organs and partial abortion of the female is in some way associated with the presence of an extra chromosome, while in *Oenothera semilata* an extra chromosome is present, but only a portion of the pollen grains abort. Morgan has shown that slight mutations are continually occurring in *Drosophila* spp., which inhibit the development of the 2X individual, while Bridges (*5*) has shown that certain chromosome combinations can not bring about normal development in the zygote. Is it too much to expect that like conditions may affect the 1X generation also?

Dorsey (*12*) pointed out a different type of pollen degeneration from that which expresses itself in the production of empty grains. He showed that in functionally pistillate grapevines pollen developnemt proceeds normally through the microspore division and the formation of the normal content of cytoplasm found in mature fertile grains. During the period of development following the microspore division, one or both of the nuclei of a portion of the pollen grains aborted, leaving the grain normal as far as cytoplasm was concerned. Associated with the complete sterility of the pollen of the functionally pistillate varieties were the reflexed type of stamens, an entire lack of sutures and germ pores in the mature pollen, and dieciousness. It is probable that the lack of germ pores is the direct cause of sterility in the numerous grains which otherwise appear entirely normal. Tischler, Rosenberg, and others have shown that pollen abortion in hybrids may follow either normal or irregular reduction divisions, when the parents have both an equal and unequal number of chromosomes. Shull (*35*, *36*) has recorded some consistent irregularities in sex ratios in *Lychnis dioica* which will later be shown possibly to have been due to slight mutations causing pollen abortion. Goodspeed (*21*, *22*) gives further evidence on the sterility of hybrids of *Nicotiana* spp,,when *N. sylvestris* is used as one parent and shows that not only the pollen is sterile but that the F_1 plants are incapable of forming any very appreciable amounts of seed. Rimpau (*31*) and Jesenko (*26*) have shown that in hybrids between wheat and rye there is complete male sterility, while some of the egg cells are able to produce viable seed if pollinated with either wheat or rye pollen.

The cytological investigation of pollen development in the strawberry, reported in this paper, has two main objects in view: (1) the determination of the mechanism and nature of pollen abortion and with these facts at hand, (2) the determination to which of the many categories of sterility the very prevalent pollen abortion in the strawberry varieties belongs.

MATERIAL AND METHODS.—The variety used as the basis of this study is Minnesota 3. As was previously stated, it furnishes desirable material for this type of study, as about 50 per cent of its pollen aborts, while the remainder develops normally. The egg cells in this variety and in the cultivated varieties in general do not show a corresponding

degree of abortion, as it is very common for practically all of the achenes to develop on perfectly formed strawberries.

The material was prepared for microscopic examination according to the ordinary cytological methods. Carnoy's, Flemming's strong, medium, and weak, and chromacetic-acid fixing solutions were used. All gave very good results, except Carnoy's fluid. Sections were cut from 4 to 20 μ thick, the best results being obtained from those 4 to 6 μ thick. The triple stain and Haidenhain's iron-alum-hymatoxylin stains were used, both giving good satisfaction.

The drawings in Plates B to E were outlined with the help of an Abbe camera lucida. All, with a few exceptions noted, are drawn to the same scale, in order that comparisons of cell size and cytoplasm content may be readily made.

ANTHER TISSUES.—The walls of the young anthers are made up of four oblong layers of cells of about equal size; the outer epidermal layer and three inner layers. Inside of these layers there is usually one layer of tapetal cells and about five layers of pollen mother cells, both of which at this time are easily distinguished from the wall cells by their large size and different staining reaction.

The growth and development of the wall layers should be followed because of its relation to the increase in size of the pollen mother-cell cavity during the formation of the tetrads. As the pollen mother cells prepare for reduction, and during the division, rapid cell division is taking place in the parietal cells, so that by the time the heterotypic division is complete there is an appreciable increase in the size of the anther cavity. Gradually the two inner layers of cells flatten out, owing probably to the growth of the outer layer, the cells of which rapidly increase in size, although showing no further cell divisions.

At the tetrad stage the inner layer is very much flattened, while the middle layer is still plainly visible and the cells are still full of cytoplasm. The cells of the outer layer are now very appreciably larger than the epidermal cells, which have also grown slightly. After the liberation of the microspores and while they are increasing in size, the epidermal cells sometimes collapse, as their contents have become scant. By the time the microspores have nearly completed their growth and have begun to divide, the cells of the outer layer have become deeper than long and are nearly as large as the tapetal cells. At this time they show distinctly the spiral thickenings which have to do with dehiscence. Both of the inner layers have now collapsed or show very scant cytoplasm. Before dehiscence the walls separating the members of the two pairs of loculi break down, leaving two large loculi in each anther. The relation between the increase in size of the anther cavities both during preparation for the first meiotic division and subsequent to it, and a difference which *Fragaria* spp. shows from some other forms in the history of the mother-cell wall will be pointed out later.

The tapetum.—The tapetum, usually one cell layer thick, is composed of large angular cells similar in size, and staining reactions to the pollen mother cells. The tapetum in *Fragaria* spp. differs in its greater persistence from most other forms reported. Division in the tapetal cells begins at about the time of synapsis of the pollen mother cells and has been observed as late as the metaphase of reduction division. The divisions are all mitotic, no evidences of amitotic divisions having been observed. Following reduction division the tapetal cells are binucleate and remain so until the liberation of the microspores, when they degenerate and completely disappear. The disappearance of the tapetal layers is gradual. They first separate from the wall layers and then proceed to dissolve, the wall which was in contact with the anther wall first disappearing, followed by gradual dissolution of the entire cell layer. During this process the walls become thick and laminate and in places the middle lamella dissolves, partially freeing the individual cells. When the microspores are three-quarters grown the tapetal cells have entirely disappeared. This persistence of the tapetal cells will be shown to be correlated with a like persistence of the pollen mother-cell walls. The history of the tapetum in *F. virginiana* is identical with that just described which is of Minnesota 3.

Pollen mother cells.—The study of pollen development was begun with the so-called resting stage of the pollen mother cells between the last archesporial division and the first meiotic division. They do not, however, show the characteristic chromatin and linin condition found in true resting cells of *Fragaria* spp. (compare fig. 1, Pl. B and fig. 2, Pl. D). The cells are angular and contain a large nucleus (Pl. B, 1). The contents of the nucleus are irregular dark-staining, very small masses of chromatin held in a network of linin fibers. The number of chromatin bodies is very much larger than the number of chromosomes. Ordinarily one large nucleolus is present, although it is not uncommon to find two.

Synapsis.—The first indication of the onset of the prophase is to be seen in the gradually increasing size of these chromatin bodies which still, however, appear very flaky and irregular. The linin and chromatin during this period are so indistinct that it is impossible to determine whether there is any definite pairing of the individual particles and threads as has been shown to exist in *Lilium* spp. by Allen (*2*) and in several forms by Overton (*30*). These larger masses gradually move to one side of the nuclear cavity and congregate about or near the nucleolus in a loose indefinite mass (Pl. B, 2). At this time a few rather definite threads appear in the mass, some extending out from it as loops. Where the loops are long enough, they are seen to be distinctly double (Pl. B, 2, 3). This is the only evidence of any pairing during the presynaptic stages. Gradations between the conditions shown in figures 1 and 2 occur within a single loculus of an anther and are proof

that these stages bearing bivalent loops are presynaptic. Gradually the synaptic mass tightens until it is close and compact, occupying a very small portion of the nuclear cavity (Pl. B, 4). During this contraction there are refractive particles present both in the mass, some of which are in contact with the nucleolus, and outside of the nuclear membrane, which give the same staining reactions as the nucleolus. Similar masses to these Digby (*11*) has considered to be synaptic extrusions.

The synaptic stage is of long duration. Gradually the chromatin in synapsis takes on the appearance of being made up of a closely tangled mass of threads. Soon loops are pushed out from it, which are bivalent often for their entire length (Pl. B, 5). The fact that these loops are often double from the point at which they leave the mass to the point of entrance, and can sometimes be traced through a portion of the synaptic mass gives the impression that they are made up of two continuous threads which closely approximate each other over their entire length. This view is supported by the later stages, especially those at and following segmentation, which in *Fragaria* spp. are very clear.

Loops continue to push out from all sides of the synaptic mass, often shifting it to the center of the nucleus. Gradually the bivalent thread becomes more or less regularly distributed about the nuclear cavity, usually having, however, a somewhat tangled center near the nucleolus. The spireme thread at this stage often appears to be a single strand due to the close approximation of its univalent portions (Pl. B, 6). However, no anthers at this stage of development have been found which do not contain many portions of the spireme which are double for considerable distances. It is probable that, during the post synaptic stages up to segmentation of the spireme into chromosomes, the univalent portions of the thread are never fused throughout their whole length to form a single spireme. It is even possible that no fusion takes place, but that the univalent threads only approach each other so closely that in such delicate threads the line of demarcation can not be distinguished.

There is no distinct second contraction, but there is a semblance of one following the loosely-distributed spireme stage. The thread contracts gradually but unevenly throughout its whole length, its univalent portions as a consequence becoming separated from one another and appearing thicker (Pl. B, 7). The portions of loops which are in contact with the nuclear membrane remain so and often extend long distances on the periphery and then turn at relatively sharp angles and again extend in fairly straight lines toward the central mass, still situated usually near the nucleolus. Many of the loops in this way form equilateral triangles. There is no doubt at this time of the double nature of these loops. The paired threads are evidently identical with those which appeared while passing out of synapsis and may be identical with the bivalent loops seen extending out from the loose presynaptic

mass. Continued contraction of the bivalent spireme results in segmentation.

SEGMENTATION.—In order to determine definitely whether a telosynaptic or a parasynaptic arrangement of the univalent chromosomes prevails during the synaptic and postsynaptic stages, it would seem necessary to determine the exact procedure from the bivalent condition just previous to segmentation, through segmentation to the paired condition in diakenesis. If it can be shown that the bivalent threads appearing during the pre and post segmentation stages are identical in *Fragaria* spp., it will be a strong argument in favor of the parasynaptic arrangement of the chromosomes. As *Fragaria* spp. is not complicated by a second contraction and, as the segmentation stages are rapid, all stages from that shown in Plate B, figure 7, to diakenesis being found within a single loculus; and as the stages during this period are unusually distinct, such a determination is not difficult.

Digby (*11*) has recently presented the results of a very detailed study of the cytology of *Crepis virens* in which the conclusion is reached that there is an end-to-end arrangement of the chromosomes during the synaptic stages. The details from the loosening of the synaptic mass to segmentation are very similar to those in *Fragaria* spp., although the chromatin in *C. virens* is apparently much more viscous and gives less clear-cut images than does *Fragaria* spp. Her figures 76, 78, and 79 may be considered as in the same stages as my figure 7 of Plate B and to present the same condition—that is to say, a split or a double spireme the pairs of which are somewhat twisted about one another. She considers that these figures do not show two univalent threads lying side by side, but that the bivalent loops are due to the folding back upon each other of univalent segments during second contraction. At this stage the chromatin mass became so viscous that—

> it is generally impossible to individualize the future three bivalent chromosomes. The chromosomes are in fact evolved out of what appears to be a tangle of viscous nuclear contents.

It appears hardly logical to conclude from this evidence that there is an end to end rather than a side to side pairing of the univalent chromosomes.

The number of loops present at segmentation in species of Fragaria is always less than the number of chromosome pairs which appear at diakenesis. It has been mentioned that these loops extend for long distances on the periphery of the nucleus, forming more or less regular equilateral triangles. When segmentation takes place, it is usually at the outer angles of these loops and at or near the nucleolus, which generally forms more or less of a center from which the loops radiate. Thus the bivalent loops are often divided into three pairs of bivalent chromosomes (Pl. B, 9). The pairs continue to contract, those attached to

the periphery remaining in that position and those which have one end attached to the nucleolus assuming a position alongside of it (Pl. B, 11, 14). Occasionally one pair may be attached both to the nucleolus and to the periphery; when there is evidence of considerable force exerted by the pair in contraction (Pl. B, 11). The pairs continue to contract (Pl. B, 12, 13), forming various figures which have often been described in other forms, but very few circles have ever been seen at this time. The contraction continues until the typical diakenesis stage is reached when it is often difficult to distinguish between the two univalent chromosomes of a pair (Pl. B, 14, 15). Apparently they often fuse, as in the multipolar spindle stage they sometimes appear as single entities. At diakenesis 26 chromosome pairs can readily be counted. At this stage in Minnesota No. 3, of 22 counts made, 19 showed definitely 26 chromosome pairs and three others showed, respectively, 24, 25, and 27.

A very similar condition to the diakenesis of the pollen mother cells is shown in the prophase of the tapetal cell divisions. Here, however, in place of the 26 pairs of chromosomes 52 pairs appear arranged about the periphery of the nucleus. Five counts made at this time showed in three cases 52, and in two 50 and 54 pairs, respectively.

Heterotypic division.—Diakenesis in the pollen mother cells is of somewhat long duration, but the period between it and the metaphase of the heterotypic division is extremely short, usually not more than two or three multipolar spindles appearing in a loculus simultaneously. The small oval chromosomes now arrange themselves on the equatorial plate. Whether there is any definite order or arrangement could not be determined, as the chromosomes appear identical. They are arranged close together and, while their number can not be readily determined, 26 have been counted on one plate and 24 on another.

The chromosomes are then gradually pulled apart and drawn to the poles. No irregularities in cell division or extrusion of chromatin matter have been seen during this process. The daughter chromosomes show only slight evidence of fission for the following division. The disk-shaped daughter nuclei are soon formed (Pl. C, 1), and directly after prepare for the second meiotic division.

Homeotypic division.—The two spindles of the homeotypic division may be parallel to one another or their axes may be at right angles. The metaphase of the division is also characterized by great uniformity, the daughter chromosomes separating and advancing toward the poles simultaneously. After separation they could be readily counted and showed in 7 counts 26 chromosomes (Pl. C, 2).

The daughter nuclei are soon formed, and walls are laid down between them, dividing the cytoplasm evenly. The cells gradually split apart, separating the four microspores and allowing the entrance between them of the viscous material which has up to this time surrounded either

partially or entirely the original mother cytoplasm (Pl. B, 9; Pl. C, 1, 3, 4).

POLLEN MOTHER CELL WALL.—The history of the pollen mother cell wall is of interest as it differs somewhat from that generally reported for the higher plants.

In the lily (*Allen, 1*), grape (*Dorsey, 12*), and in many other plants in which pollen development has been studied, it is usual, during preparation for the first meiotic division, for the pollen mother cells to separate from one another, due apparently to dissolution of the middle lamella and to growth of the anther walls, forming a greater space into which the cells can round up and float free from one another. Allen (*1, p. 200*) considers that the separation is due to a dissolution of the cell walls from between the mother cells, and that each is "surrounded only by a plasma membrane." Following separation, a very decided thickening of the material surrounding the cytoplasm takes place (Pl. B, 9; Pl. C, 1). This, Allen (*1*), Tischler (*41*), Stevens (*38*), and others speak of as a thickening of the mother cell wall. Following the formation of the tetrads, this material increases and, as the cells of the tetrad separate from one another, flows between them. This material is usually of a rather firm nature and in buckwheat (*38*) often persists for some time after the liberation of the microspores from it.

There is evidence in *Fragaria* spp. which indicates that this material is entirely distinct from the mother cell wall and is in no way dependent on it for its increase in volume, thus appearing to be more of the nature of the gelatinous sheath which surrounds groups of cells in many of the algae.

In *Fragaria* spp., in place of the pollen mother cells rounding up just before or during reduction division, while there is taking place a rapid growth of the anther walls and a consequent increase in size of the anther cavities, the cytoplasm separates at the angles from the walls and rounds up independently (Pl. B, 9, 14, 15; Pl. C, 1). The walls remain in contact with one another and adjust themselves to the increasing space by stretching. As soon as evidence of rounding up of the cytoplasm appears, a gelatin-like material is secreted unevenly about the cytoplasmic mass (Pl. B, 9; Pl. C, 1). This material is apparently identical with that laid down about the plasmic mass in the grape, lily, and forms like them in which the mother cell wall rounds up, supposedly following the dissolution of the mother cell wall. In these cases it is generally spoken of as the thickening of a new mother cell wall. In the strawberry this material increases in amount until at the completion of tetrad formation and before liberation of the microspores, the spores are completely embedded in it (Pl. C, 3, 4). The mother cell walls are still present, but simply divide the anther cavity into large spaces, which are only partially filled by the tetrad (Pl. C, 3). An examination of analogous stages to these in the lily and

grape shows the mother cell wall lying closely about the gelatinous material in which the spores are embedded, it having not disappeared at the period of rounding up of the pollen mother cells, as is generally assumed. At the time of rounding up of the pollen mother cells in these two plants, there is no evidence of old walls being left behind, but there seems rather to be a separation of the contiguous walls, due to the dissolution of the middle lamella and rounding up of them with the plasma masses. The walls give a slightly different staining reaction from the thick secreted mass and so can be readily distinguished from it. During the liberation of the spores in the grape, it is not uncommon to see this gelatinous sheath completely disappear, leaving portions of the original mother cell wall about the spores. This gradually dissolves, liberating the spores. In the strawberry, the procedure is much the same. The gelatinous sheath, which shows no wall closely about it but only a limiting membrane, dissolves, liberating the spores into the large cell bounded by the mother cell wall. This soon disappears.

At the time of liberation the spores have a distinct wall about themselves, which is independent of the surrounding sheath. Apparently the condition in the lily, grape, and strawberry is identical, as far as structures are concerned, but differs primarily in the separation of the walls of contiguous cells in the former plants, while in the latter only the cytoplasm of the pollen mother cells rounds up, leaving the walls and middle lamella intact.

Following the liberation of the tetrads and the disappearance of the mother cell walls, degeneration of the tapetum takes place. Tapetal degeneration seems to be in some way correlated with the disappearance of the middle lamella from between the mother cell walls, for in those forms which show an early rounding up of the mother cell walls there is a correspondingly early degeneration of the tapetal cells.

Growth of the microspores.—Following the liberation of the microspores, there is a period of very rapid growth in their walls. At first this causes the microspores to become very irregular in outline (Pl. C, 7), but as growth continues and the wall becomes thicker the cells become more spherical. The growth of the microspore wall is so rapid that there is not a corresponding growth of the cytoplasm, a condition which results in a large vacuole occupying the greater portion of the cell cavity. When growth of the microspores is nearly complete, the original cytoplasmic content of the spore is spread out over the periphery of the cell and about the nucleus (Pl. C, 8). The comparative size and consequently the very great decrease in relative cytoplasmic content, of the newly liberated microspores and those ready for the microspore division may be realized by comparing figures 5 and 8 of Plate C, which are drawn to the same scale.

Early in the development of the liberated microspore the wall is differentiated into the extine and intine (Pl. C, 7). The extine gradually

thickens and a series of scales are formed over its surface. The external appearance of the extine is shown in Plate C, figures 15 and 16; Plate D, figures 6 and 15. Soon after the microspore division the extine development is practically complete.

Division of the microspore nucleus.—With the growth of the microspore there is an apparent decrease in chromatin content; for, in the nuclei which are just about to divide, the chromatin is distributed in small particles about the periphery of the nucleus and appears very scant. Transition stages between that seen in Plate D, figure 8, and the completion of the spireme have not been observed. The spireme (Pl. D, 9, 10) is a continuous, heavy, dark-staining thread. It follows, more or less, the periphery of the nucleus and surrounds the large nucleolus which has at this time begun to break down. The nucleolus takes on a very irregular outline, which is in some way related to the numerous threadlike processes which extend from it to the spireme thread (Pl. D, 10). The nucleolus is now very light-staining and seems for the most part homogeneous, but it may contain one or more small vacuoles. Soon after the segementation of the spireme into its 26 constituent parts the nucleolus completely disappears.

Following segmentation of the spireme the nuclear membrane disappears, the chromosomes are drawn to one plane, and the spindle is formed (Pl. D, 11). The most usual position of the spindle in the microspores of many plants is near the wall and perpendicular to it; the pole which is to form the generative nucleus being nearly in contact with it. As a result of this position the generative cell usually lies against the wall and the vegetative nucleus in the cell cavity (Pl. D, 15). This arrangement of the spindle is also found commonly in *Fragaria* spp., but is not universal. Often spindles are found which lie parallel to the wall (Pl. D, 12) and result in the arrangement of the nuclei shown in Plate D, figure 14, both of which lie against the wall.

The spindle is always broad at the poles and short. The chromosomes on the equatorial plate are small and oval in shape and may readily be counted if a section can be obtained in which the heavy wall of the microspore is removed both above and below the chromosomes. In these sections 26 chromosomes are plainly visible. The daughter chromosomes are drawn to the poles simultaneously (Pl. D, 12), no instances having been seen in which chromosomes lagged behind on the spindle or in which there was an extrusion of chromosomes.

Directly after the rounding up of the daughter nuclei and the disappearance of the spindle fibers there is visible no sign of a cell wall or limiting membrane (Pl. D, 13) between the two nuclei, which is eventually to set off the generative nucleus in a separate cell (Pl. D, 14, 15). Soon, however, the wall appears and the generative cell begins to round up (Pl. D, 14), eventually to lie free in the cytoplasm of the pollen grain (Pl. C, 1).

Pollen maturity.—After the microspore division there is again a period of growth of the pollen grain and a very marked increase in the amount of cytoplasm. Eventually the pollen grain is completely filled with cytoplasm of a distinctly alveolar structure. During this time the cytoplasm nearly disappears from about the microspore nucleus, leaving the microspore cell wall loosely surrounding the nucleus, the chromatin of which is gradually taking on the condition typical of resting nuclei (Pl. D, 2).

When pollen formation is complete, changes take place in the anthers preparatory to dehiscence. These consist primarily in breaking down the wall between each pair of loculi, thus throwing all of the grains of one-half of the anther together. There is also a general drying-out process which results in the disappearance of the liquid material which previously surrounded the developing grains, and of a considerable portion of moisture from the pollen grains, thus causing them to collapse along three meridial sutures which fold in, thus giving in cross section somewhat of a clover-leaf shape, while the general shape of the grain is long-oval. The three germ pores are located at the middle points of the sutures. On placing the dry pollen in water it soon swells to form a sphere.

The position of the generative cell and vegetative nucleus within the collapsed grains could not be determined, for as soon as the killing fluid penetrated the anthers the grains immediately swelled. In the swelled grains the nuclei lie in various positions with regard to one another, but usually in close proximity. The vegetative nucleus is generally spherical while the generative cell contents are fusiform, owing to the folding of the dried pollen grains and are not closely surrounded by the cell wall. The chromatin of both nuclei now shows the typical resting condition (Pl. D, 2).

Up to and including the liberation of the microspores from the tetrad, the cells have shown marked regularity in division, the stages of short duration proceeding regularly from one end of a loculus to the other, while during the stages of longer duration the cells of a loculus all show the same degree of development. Up to the time of liberation of the microspores, the development which has taken place has depended entirely on materials furnished by the sporophyte, the one group of chromosomes merely being the tools by which the materials of the pollen mother cell were divided into four parts, the microspores. So far as can be seen up to this point, no growth process resulting directly in an increase of cell material can be directly attributed to the one aggregation of chromosomes. At the time of liberation from the tetrad the spores are strikingly alike in size and all other visible characters. There has been, up to this time, no differentiation in rate of development of individual microspores; and the spores, as liberated, are all normal.

DEGENERATION OF THE MICROSPORE

Liberation of the microspore from the tetrad places these individuals upon their own resources for future development. It is true that the spores are surrounded by a nourishing medium furnished by the sporophyte, but the ability to use this material depends upon the individual cell metabolism of the microspores.

A study of the actual change in cytoplasmic content from the pollen mother cell stage to the stage of complete formation of the pollen grains indicates the degree to which the young spores are dependent upon their own metabolism from the time of liberation from the tetrad to maturity of the male gametophyte or mature pollen grain. Measurements were made of the diameter of the spherical cytoplasmic mass at the following stages during pollen formation: Rounded pollen mother cell at diakenesis, young microspores which have just been liberated from the tetrad, microspores which have completed growth and in which the nuclei are either dividing or are about to divide, and the mature pollen grains. A summary of the measurements is given in Table XI. It will be seen by comparing the volume of the pollen mother cells and of the microspores at liberation that the latter show slightly less than one-fourth the volume of the former, indicating that during reduction division and the period subsequent to the liberation of the microspores no increase in cytoplasm has occurred. Following liberation from the tetrad, however, when the microspores are floating in the nourishing medium of the loculus as independent units, very rapid wall growth takes place. From the time of liberation to division of the microspores nucleus the volume of the cells increases about 6.4 times, but shows no corresponding increase in cytoplasm. At the mature pollen stage the cells have increased to nearly 7.5 times that of the liberated microspore. The changes in volume and cytoplasmic content from the pollen mother-cell stage to mature pollen are also well illustrated by Plate B, figure 14; Plate C, figures 4 and 8; and Plate D, figure 2, all of which are drawn to one scale.

TABLE XI.—*Volume of pollen mother cells and microspores at various stages of development*

Stage.	Number measured.	Average diameter.	Volume.
		μ.	*Cu.* μ.
Pollen mother cell at diakenesis	263	15.25	1,859.99
Liberated microspores	300	9.41	436.28
Microspores, division stage	200	17.44	2,777.40
Mature pollen	500	18.39	3,255.39

Although the gametophytic generation must properly be considered as beginning with the first appearance of the haploid chromosome number, yet the liberation of the spores from the tetrad may be considered as marking the beginning of the independent growth period of this generation and the rapid growth of the spore wall, division of the microspore nucleus, the increase in cytoplasm, and finally the germination of the pollen grain and the production of sperms as developmental stages in this very much foreshortened plant generation. The very rapid wall growth, division of the nucleus, and finally the increase in cytoplasm to 7.5 times its original volume, all within a relatively short period, go to make this period of a plant's life history probably the most finely adjusted and critical one which it is called upon to survive.

It is during the periods of rapid enlargement and of increasing cytoplasm that degeneration of the microspores takes place.

Although there is some evidence in the strawberry that degeneration may begin before liberation of the microspores from the tetrad, so many anthers have been examined which contain only normal microspores, both in the tetrad stage and directly following their liberation, that degeneration before this time can be considered as negligible. It is probable that poor fixation may account for the few apparent instances which have been found. Indeed, poor fixation constantly enters as a disturbing factor in the interpretation of the condition of the supposedly aborting grains, and it is only by finding the same types at later and more advanced stages of degeneration when there can be no question as to the condition of the microspore that the factor of poor fixation can be eliminated. No aborted microspores have so far been found which could be referred to as degeneration within the tetrad.

Directly following microspore liberation evidences of pollen abortion may be noticed and from this period on through the various stages of development, microspores and pollen grains are continually aborting.

Plate D, figures 3 and 4, shows microspores shortly after liberation, in which the contents have completely broken down into a yellow oily mass which turns black on exposure to osmic acid. The lighter areas are globules evidently of a different substance.

Ordinarily during the growth period of the wall following liberation, the cytoplasm becomes spread over the periphery in a thin layer. Plate D, figures 5, 6, and 7, represent various conditions in which this normal process has not occurred. Figures 7 and 11 are later stages of this type of abortion in which degeneration of the nucleus and cytoplasm is gradually taking place. Figure 15 apparently represents a further stage of this series, in which the nucleus, although visible in outline, is, with the cytoplasm, entirely functionless. Plate E, figure 11, shows the completely degenerate microspore of this type found among normal mature pollen. The dark-staining mass is yellow and oily before killing.

Although there has been a considerable amount of degeneration up to the time of the completion of growth of the microspore wall, probably more takes place between the time of the formation of the large vacuole (Pl. C, 8) and the completion of the microspore division than at any other period.

Plate D, figure 12, shows a full-grown microspore in which degeneration is taking place, both in the cytoplasm and in the nucleus. This was found in an anther containing full-grown 1-nucleate microspores, and probably is a case in which degeneration has begun during the period of enlargement.

Plate D, figure 13, and Plate E, figure 2, represent early stages in degeneration of 1-nucleate microspores which are at the stage at which division should take place as they were found in anthers containing both 1- and 2-nucleate grains, as well as division stages. The microspore represented in Plate E, figure 7, was found among microspores of the stage of development shown in Plate C, figure 15, while that of Plate E, figure 8, was found among mature pollen grains. Both are evidently later stages of the condition represented in Plate D, figures 12 and 13, and Plate E, figure 2.

During the period of division a few microspores contain a very scant amount of cytoplasm. Such spores are seen in Plate D, figures 14 and 16, and Plate E, figures 1 and 4. Eventually these completely degenerate. Those of the type shown in Plate D, figure 16, and Plate E, figure 1, probably are the forerunners of the completely degenerate microspore shown in Plate E, figure 10, which is a common type in mature anthers.

Although degeneration takes place to a greater or less degree at all stages, from microspore liberation to microspore nucleus division, no instances of degeneration occurring during the process of division have been noted. Directly following division, however, evidences of degeneration again become apparent, although much less numerous than in the period just prior to division. Figure 3 of Plate E represents an early stage of degeneration directly following microspore division. This is probably an early stage of the more advanced degeneration stage shown in Plate E, figure I. This young pollen grain was found among grains in which the cytoplasm was increasing rapidly in amount. It will be noticed that the generative cell is aborting, while the remainder of the grain is normal. Apparently grains of this type continue to increase normally with regard to the vegetative portion, while degeneration of the generative cell occurs. Figure 13 of Plate E represents such a grain found in a mature anther. Figure 12 represents another type of degeneration which takes place subsequent to division of the microspore nucleus. The generative cell has completely degenerated. The vegetative nucleus, although still present in outline, is functionless, while the cytoplasm bears no resemblance to the normal. It still retains the property, however, of

absorbing liquids when placed in them, and so the grains of the type shown in Plate E, figure 13, are likely to cause inaccuracies when the ordinary methods of determining the percentage of good and abortive pollen are employed.

It seems clear thus far that degeneration of the microspores and pollen grains is a phenomenon closely related to the very active metabolic processes which are taking place during this period of the plant's life history.

DISCUSSION OF RESULTS

Thus far there has been shown to exist in the strawberry types of sterility due to at least two distinct causes.

In some of the wild species, including *F. elatior*, *F. platypetala*, *F. cuneifolia*, and *F. chiloensis*, it seems highly probable that the species are diecious, while *F. virginiana* is unquestionably so for the most part. Dieciousness is expressed in the production of pistillate plants bearing staminodia, which, so far as I have observed, never produce pollen. The staminate plants bear normal stamens and pistils, which appear normal but which seldom are fertile. Certain types are also found which are intermediate between these two types. A few staminate plants may bear fruit on one or more of the early flowers. These flowers may or may not bear stamens, but both staminodia and intermediate anthers are found on them. Other plants which are apparently staminate develop only intermediate anthers in which abortion takes place at the tetrad division or shortly after, resulting in a degenerate mass in the anther. Another staminate type has recently been studied which bears fruit on the primary and occasionally on some of the other flowers. The anthers of the primary flowers are reduced to staminodia. The secondary flowers carry pollen development through to the liberation of the microspores when 40 to 80 per cent of them abort and degenerate completely, forming a yellow oily mass. The remaining grains develop normally and are fertile. These types are all found in plants of pure *F. virginiana* and represent varying degrees of expression of dieciousness.

The diecious condition of the wild species from which the cultivated forms have been derived probably explains the greater sterility of the later flowers of the cultivated hermaphroditic varieties, than is found in those of the pistillates; if we can accept the origin of the hermaphrodites as being from males which have developed partial fertility of the female organs. The appearance of staminodia showing varying degrees of development in the cultivated varieties is also the direct result of dieciousness, while the intermediate types of anthers in the cultivated forms are of the same nature as those found in wild staminate clones. It is an interesting fact, in connection with the problem of sex determination and dieciousness, that where intermediate anthers or staminoids occur, either on wild clones or cultivated varieties which are able

to bear normal anthers, they are practically always borne on the primary flowers, while the anthers produced later have a greater tendency toward normal development. The tendency toward the production of staminodia is much greater in the early spring than later. On the other hand, pistil sterility is much more frequent on the later flowers of an inflorescence and when fruits set on wild staminate clones it is practically always on the first flowers of a cluster which open.

A second type of sterility often associated with the above type but due to a different cause is that which results in aborted microspores and pollen grains in otherwise normal anthers. Aborted pollen has been shown to be present in relatively small amounts in pure species of Fragaria, but appears often in large quantities in many of the cultivated varieties. This type of abortion has long been recognized in hybrids, and recently Jeffrey and his students have gone so far as to consider any plant bearing over 15 or 20 per cent of this type of pollen a hybrid.

Selfed seedlings and F_1 plants of crosses between varieties of cultivated strawberries are so extremely variable for many factors that it seems self-evident that they are of hybrid origin, and this is to a great extent confirmed by what is known of the origin of the numerous cultivated varieties, many of which are the result of variety crosses, while by far the larger number are chance seedlings. It thus seems evident that pollen abortion in the cultivated varieties is due to the same causes which produce sterility in other hybrids.

As would be expected, there are varying degrees of sterility resulting from hybridization and varying degrees of irregularities in the stages which lead up to the final abortion of pollen. There appear in the literature numerous instances of abortion in both male and female reproductive organs following irregular reduction divisions. The irregular divisions, especially in the pollen mother cells, result in the production of more than four cells of unequal size in the tetrad. These produce microspores of varying sizes, few of which ever come to maturity. Gates (*17, p. 98*) pointed out that most of the forms studied by Wille (*44*) showing supernumerary cells in the tetrad are either hybrids or have been under cultivation for some time and are open to the suspicion of being hybrids. Other plants, some of which are known to be hybrids while others which have been cultivated as horticultural varieties and are under suspicion as hybrids, have been studied in more detail by various workers. Tischler (*40, 41, 42*), who has done much work with plants of this type, finds that in hybrids of *Ribes* spp. and in the sterile hybrid *Mirabilis jalapa* × *M. tubiflora* pollen degeneration usually takes place following normal divisions. In the hybrids *Potentilla tabernaemontani* × *P. rubens*, *Syringa chinensis*, and *Bryonia alba* × *B. dioica*, and in three varieties of banana (*Musa paradisiaca*) having different chromosome numbers, irregular divisions are common and are always followed by much pollen abortion. In these banana varieties, the

origins of which are unknown but which differ cytologically in having 8, 16, and 24 chromosomes as the reduced number, it is significant that the most frequent irregularities in cell division during reduction, and most complete pollen sterility occurs in the two varieties having the greater chromosome numbers. Thus, pollen abortion may or may not be the result of irregularities at reduction division, but is apparently related to hybridity and is associated with heterozygosity.

After working with several hybrid plants showing both normal divisions and irregularities during reduction and formation of tetrads, Tischler (*41, p. 144*) concluded that—

Die Sterilität bei Hybriden hängt nicht von irgendwelcher Chromatin repulsion ab.

He concluded further that irregularities during tetrad division can not be considered as characteristic only of hybrids. He thought that sterility of hybrids was due to the coming together of two sex cells which did not contain identical developmental tendencies and that these were expressed at the critical time of the formation of the reproductive organs. Actual abortion of the grains he thought was due to insufficiency of cytoplasm in the enlarged microspores.

One of the most striking cases of sterility following hybridization, the cytological details of which have been worked out, is that of the hybrid *Drosera longifolia* × *D. rotundifolia*, reported by Rosenberg (*33*). The striking feature of this hybrid is that it is between parents having different chromosome numbers, the diploid number of *D. longifolia* being 40, while that of *D. rotundifolia* is only 20. As a consequence the hybrid contains 30. At reduction division in both the megaspore mother cells and pollen mother cells there appeared 10 pairs of chromosomes and 10 single ones, the pairs supposedly being made of the 10 *D. rotundifolia* chromosomes paired with 10 from the *D. longifolia* parent, while the 10 single chromosomes were the remaining 10 *D. longifolia* chromosomes. Reduction division resulted in the separation of the paired ones, these being drawn regularly to opposite poles. The unpaired chromosomes, on the other hand, were either drawn to one or the other pole or were left in the cytoplasm to form another small nucleus. The homœotypic divisions took place normally. Following the organization of the microspores within the tetrad, many proceeded to increase in size; in some, division of the microspore nucleus proceeded normally, and then in practically all cases abortion of the pollen took place. Following tetrad formation in the female reproductive organs, three of the tetrads usually aborted, as is common, while the other proceeded to form the egg sac. Egg-sac formation was carried to various stages, but it was only very rarely that a perfect egg sac, capable of further development, was formed. Rosenberg concluded (*p. 39*) that because of the fact that the microspore division was able to proceed normally, the degeneration of the pollen grains was not the result of the irregular distribution of chromo-

somes during reduction division, but was due to a lack of cytoplasm. The abortion of the egg sacs, he again concluded, was not due to irregular divisions, as all of the divisions following reduction were normal, but was due to poor nutrition.

Nakao (29), working on the cytology of certain grain hybrids in which very striking irregularities in reduction division took place, followed by complete abortion of the microspore after liberation, concluded that abortion in this case was due to an insufficiency of cytoplasm previous to reduction division which resulted in abnormally early division and consequent irregularities. He did not consider why these irregularities should cause abortion of the microspores.

In view of the conditions existing within an anther at the time of degeneration of microspores and because of certain genetic results which can only be explained on the basis of selective elimination of certain gametic combinations, it is difficult to agree with the conclusions of Tischler and Rosenberg that degeneration has nothing to do either with the irregularities or normal repulsion which occur at reduction division in hybrids.

Although there are striking differences in the regularity with which reduction takes place in sterile or partially sterile hybrids, there are certain conditions, in both those which proceed normally and those which show irregularities, which are alike and must be taken into account in the consideration of the causes of pollen abortion. These conditions are as follows:

(1) At reduction division there is a sorting out of the parent chromosomes, resulting in new combinations in the daughter cells, the mnuber of which depends upon the degree of difference between the two parents. If division proceeds normally, there is an equal number of chromosomes in each daughter nucleus. If it takes place irregularly, unequal numbers are found in the resulting daughter cells. In either case the combinations are new and may or may not contain all of the properties necessary for perfect metabolism of the cell.

(2) If divisions take place regularly, there is an equal quantitative and, as far as can be determined, also an equal qualitative division of cytoplasm between the quadrants of a tetrad. If divisions have proceeded irregularly, the cytoplasm is divided between the members of the tetrad in proportion to the amount of chromatin which they contain. In either case at the time of liberation from the tetrad, or if liberation does not take place, as in *Drosera* spp., at the period previous to enlargement, all of the microspores appear normal—that is, they contain an organized nucleus and are filled with cytoplasm. In Minnesota 3 there is at this time as great uniformity in size and cytoplasmic content of the individual microspores as is found in entirely fertile plants of *F. virginiana*.

(3) It is not until rapid growth of the microspores takes place and the necessity of active cell metabolism appears that evidences of degeneration appear. The necessity of active metabolism becomes apparent when it is remembered that the microspores increase 7.5 times their original volume during this growth period.

(4) There is no specific time at which degeneration of the grains within a single anther takes place. In most of the sterile forms thus far studied a series of degenerating stages appear from the first period of growth of the microspores to the formation of nearly mature pollen.

It is becoming more and more evident that the growth and development of plants and animals are directly dependent upon the chromosome combination which they contain. Boveri (*see Morgan, 28, p. 55*), in working with dispermic sea-urchin eggs, found that they very rarely develop normal individuals, while if separated at the 4-celled stage normal individuals often developed. This seems dependent upon the fact that in the first four cells, which are the result of a single division, the chances of one of the cells receiving at least one of each kind of chromosome are relatively high and thus, when separated, some may develop normally. On the other hand, the chances of each of the four cells receiving one of each kind of chromosome necessary for perfect development are small; and as a result the individual develops abnormally. Bridges (5) has shown that in the fruit fly *Drosophila ampelophila* certain variations from the normal chromosome combinations have a definite effect upon the development of the zygote. Zygotes containing 3X chromosomes die, while those containing 2X and a Y chromosome develop normally. Male individuals may develop which contain an X but no Y chromosome, but were found to be entirely sterile; while those zygotes which received only a Y chromosome died as did also those which received 2Y chromosomes, but no X. Zygotes containing 2Y chromosomes plus an X, however, were able to develop into normal males. Apparently the presence of an X chromosome is necessary for the development of an individual, while the presence of a Y in males is necessary if the male is to be fertile.

In plants the evidence for the dependence of development upon chromosome combinations is becoming indisputable if the Mendelian interpretation of the inheritance of factors is admitted. In F_1 progeny of hybrids, if the parents are homozygous, there is generally as much uniformity as is shown by either parent, while the F_2 progeny shows a wide range of types, often overstepping the limits of the parents. If such a variety of types with regard to hardiness, rust resistance, adaptability to various regional and soil conditions, and vigor of the individual plant are produced in the 2X generation as the result of new chromosome combinations, why is it not possible for a similar series to exist in the 1X generation with regard to the ability of the individuals to develop in a given environment?

I have already pointed out that up to the time of liberation of the microspores from the tetrad, in the strawberry, cell divisions have resulted merely in an equal division of the cytoplasm of the original mother cell between its four granddaughter cells with no evidence of any metabolic changes resulting in an increase of cytoplasm. A similar condition exists in those forms which show irregular divisions during reduction with uneven distribution of cytoplasm between the resulting cells. Liberation of the microspores from the tetrad marks the end of the period of dependence of these cells upon the 2X generation, as far as future growth and development is concerned. At this time there is no difference between the conditions surrounding the microspores of a hybrid and those of a genetically pure individual. Both groups of microspores are set free in a homogeneous anther sap to complete their own further development.

The progress of the developmental stages in plants of pure *F. virginiana* is characterized by great regularity of development of the individual microspores within a loculus with regard to rate of enlargement, time of division of the microspore nucleus, and the subsequent development of cytoplasm. In fact, there is as great uniformity shown in these stages as was shown in the stages leading up to the mature tetrad stage in either *F. virginiana* or Minnesota 3. Minnesota 3, on the other hand, shows great irregularities during this developmental period in rate of growth of the individual microspores, in the time of division of the microspore nucleus, and in the rapidity of formation of cytoplasm. This lack of uniformity is in striking contrast to the uniformity shown in earlier stages of the same plant, while the cells were dependent on the 2X individual. The liberated microspores of Minnesota 3 are strikingly uniform in size and cell contents.

During any period, following liberation of the microspore to the completion of development, microspores or pollen grains may be found degenerating. As all of the grains within a loculus are free in a homogeneous nutrient liquid, it seems difficult to believe that the variations in development can be due to anything but the individual constitution of the microspores.

Indeed, there is constantly accumulating an increasing amount of evidence which points to the continual elimination of gametes bearing certain chromosome combinations. In 1894 Millardet (*27*) reported on a series of hybrids between species of strawberry the progeny of which, he said, formed an exception to the general rule of hybrids, as the specific type of one or the other parent was always produced in the first and later generations. The specific type shown in the second generation was, with one exception, the same as that shown by its parent in the F_1 generation. Millardet mentions complete sterility in one species of cross and high percentages of sterility in the F_1 generation of some of the other combinations. Bellair (*3*) reports that in the tobacco cross *Nicotiana*

sylvestris × *N. tabacum* the F_1 generation resembled the *N. tabacum* parent and was partially fertile. From these he was able to obtain F_3 plants apparently identical with the two parents and fully fertile. The reappearance of types similar to the parents in large numbers in the F_3 generation suggests the elimination of gametes containing combinations which would result in intermediate types.

Detlefsen (*10*), working with animals, reports results obtained from cavy crosses which may readily be explained on the basis of the elimination of certain combinations in the gametes of the males. He crossed tame females to wild males. The F_1 males were all sterile. The cross F_1 female with wild male was not very successful and produced one sterile male and a sterile female. The F_1 females crossed to tame males gave sterile males of which a few produced some nonfunctional sperms. The females of this back-cross again crossed to tame males produced males showing a low degree of fertility. As this process was continued, always crossing back to tame males, the fertility of the male progeny increased as they became more nearly homozygous for the tame condition. In the sixth generation all of the males produced sperms, and 66.7 per cent of these males were readily fertile. Apparently the more chromosomes of one parent type which were present in the sperm, the greater its chance of complete development.

East (*13*) in a short abstract gives the conditions which he found in the progency of the partially sterile hybrid *Nicotiana rustica humilis* × *N. paniculata*. The F_1 progeny of this hybrid were very uniform, but only 1 to 6 per cent of the female gametes were functional, and 2 to 6 per cent of the pollen grains were morphologically perfect. In the F_2 generation some perfectly fertile plants were found, many possible F_2 combinations were omitted, many more homozygous combinations occurring than should be expected, and the parent types appeared once in every 100 to 200 plants, whereas if all of the possible F_2 combinations appeared, the parent types would be much more rare. East considered that the results might be explained on the basis either of selective elimination of F_2 zygotes or selective elimination of F_1 nonfunctional gametes. He considered further that the elimination of the nonfunctional gametes might be due to irregularities of chromosome distribution, which scheme seemed improbable; or the facts might be interpreted without this assumption if certain conditions were met which are as follows:

> If (1) there is a group of chromosomes in each parent that can not be replaced by chromosomes from the other parent; if (2) there is a group of chromosomes from each parent, a percentage of which may be replaced by chromosomes from the other parent, but where functional perfection of the gametes varies as their constitution approaches that of the parental forms; if (3) there are other chromosomes that have no effect on fertility and therefore can promote recombinations of characters in the progeny of fertile F_2 plants; if (4) a naked male nucleus entering the normal cytoplasm of the egg in the immediate cross can cause changes in the cytoplasm that will affect future reduction divisions; if (5) this abnormally formed cytoplasm is not equitably dis-

tributed in the dichotomies of gametogenesis in the F_1 generation; if (6) it follows from (4) and (5) that F_2 zygotes may be formed which are less perfect in their gamete forming mechanism than those of the F_1 generation; and if (7) the heterotypic division of gametogenesis does not necessarily form two cells alike in their viability.

In the strawberry, in which no irregular distribution of chromatin occurs, certain of these assumptions—namely 1, 2, 3, and 7—would apply in explaining partial male sterility in many partially sterile varieties, but assumptions 4 and 5 and assumption 6, which is dependent upon them, can hardly be considered applicable; assumption 4 because there is no cytological evidence that there has been any disturbance of reduction division; 5, because there is apparently equal distribution of the mother cell cytoplasm to each member of the tetrad, and 6, because there is no cytological evidence which would lead one to believe that the cytoplasm of the zygote had anything to do with the perfection of its gamete forming mechanism. In the strawberry sterility seems to be due to the inability of certain chromosome combinations to use the food material in which they are embedded in the growth and development of the liberated spore to a ripe pollen grain.

If we can accept the hypothesis of pollen abortion being due in hybrids to certain chromosome combinations affecting the normal metabolism of the microspore in its development, the question at once presents itself: Is pollen abortion the result of the presence of one particular chromosome or of certain combinations of two or more, or do all of the chromosomes play some part in it? This question can not be answered from the facts so far obtained in the strawberry because of the heterozygous condition of the material which has been used; but there is other published evidence which throws some light on this question.

Belling (*4*) has made a very careful study of partial sterility of hybrids between four species of the "bean" Stizolobium. He found that the F_1 plants, of those crosses in which the velvet bean (*S. deeringianum*) was used as one parent continually, aborted one-half of the pollen grains and one-half of the egg sacs. Of the second generation plants one-half were completely fertile and one-half partially sterile, as in F_1. The progeny of fertile F_2 plants continued to be fertile, while the progeny of the partially sterile plants were one-half fertile and one-half partially sterile. Belling explained these results on the basis of the presence in the velvet bean of the factor K, which was not present in the other three varieties. These three, however, contained the factor L not present in the velvet bean. The presence of either K or L he assumed to be necessary for the normal development of either egg sacs or pollen grains, the presence of both K and L causing abortion. We may extend this working hypothesis slightly and put it on a chromosome basis, in which case we must consider that Belling's factors K and k form one allelomorphic pair, being situated in a certain loculus of a specific chromosome in the velvet and the other three varieties of beans, respectively, and that the factors

L and l form an allelomorphic pair located in a definite but different chromosome of the three varieties and the velvet bean, respectively. Then, to follow out Belling's scheme, the presence of the chromosomes bearing the factors K and L in the same member of a tetrad causes abortion, and likewise the presence of both chromosomes lacking the factors K and L causes abortion. Belling states that abortion of the microspores takes place in the vacuolate stage and that there are no intermediates between the completely aborted and the most perfect grains, thus strengthening the idea that in this case no more than two chromosomes have to do with abortion.

Another set of studies which point to one instead of two chromosomes being the cause of pollen abortion are those of Shull (*35, 36*) on the inheritance of sex and of a sex-linked factor in *Lychinis dioica*. In the first of these studies Shull showed that very probably *L. dioica* ♀ is homozygous for the sex determining factors, while *L. dioica* ♂ is heterozygous. In crossing these forms an approximate ratio of 1 pistillate to 1 staminate usually resulted, but with nearly always a slight excess of pistillate plants, suggesting, if the females are homozygous for sex, an elimination of a portion of the male gametes bearing the determiner for maleness. In a later study he was able to show that the determiner for maleness was linked with a factor for narrow leaves while in normal plants the determiners for femaleness were linked with broad leaves. In a narrow-leaved mutant male found by Baur he showed that the determiner for femaleness as well as maleness was linked with the narrow-leaved determiner. It was as a result of the discovery of this homozygous (for leaf width only), narrow-leaf male that the factor for narrow leaves linked to maleness was able to be discovered as, being a recessive character and always in a heterozygous condition, it was hidden in normal males.

When these homozygous narrow-leaved males were used in crosses with either normal broad-leaved females or heterozygous females, there was always produced a great excess of males, the females appearing only in very small numbers. These results were apparently in contradiction to those previously obtained in which females were more abundant. Shull gave no explanation of these irregularities. They suggest, however, that there is a fairly constant elimination of certain gametes. A study of all of Shull's results, with this idea in mind, indicates that an explanation based on the elimination of certain male gametes will cover all cases of irregularity so far reported by him except the nonappearance of homozygous hermaphrodites and of heterozygous hermaphrodites containing male determiners. These two instances, if we may draw analogies between plants and animals, are of the same nature as the YY zygotes in species of Drosophila, and die (*Bridges*, *5*).[1]

[1] Shull (*35–36*) has shown that the hermaphrodites have undoubtedly been derived from males; and therefore the presence of two hermaphrodites or a male and an hermaphrodite determiner would be analogous to the presence of two male determiners.

In every other instance in which irregularities in sex ratios occurred, a male or hermaphroditic parent was used in which the condition for narrow leaves was linked with either a determiner for maleness, femaleness, or hermaphroditeness. If the irregularities were relatively slight, as was the case when normal broad-leaved males and females were crossed, maleness and narrow leaf were linked. A partial elimination of these male gametes would produce the actual results obtained. Hermaphrodites of *Lychnis dioica* acted in the same manner as the males. Hermaphrodites of *Melandrium album*, which we may assume to bear the narrow-leaf and hermaphrodite determiners linked, as they have undoubtedly been derived from males, acted in the same manner as the narrow-leaved males—that is, they produced only females when crossed to normal broad-leaved females, in place of a 1 to 1 ratio. These results can be explained on the assumtion of complete elimination of the male gametes of the *M. album* hermaphrodite, which carry the hermaphrodite mutant and its linked factor, narrow leaf, and in the case of the narrow-leaved *L. dioica* males of the nearly complete elimination of the male gametes bearing the mutant factor for femaleness and narrow leaf.

In all cases it seems that the factor for narrow leaves has an inhibiting action on the formation of the male gametes and results in the partial or complete elimination of them. Elimination in the normal males is not complete; otherwise this line would long ago have disappeared. In the mutants in which narrow leaf is linked with femaleness, elimination of male gametes bearing this mutant factor is nearly complete. Shull has also given much evidence which shows that there is also some elimination of female gametes bearing this mutant factor but to a less extent than in the males. Such partial elimination of the female gametes was shown in the cross heterozygous broad-leaved female (of the formula FBFb) by a narrow-leaved male Fbfb, which produced two heterozygous broad-leaved females, no homozygous narrow-leaved females (the nearly complete absence of both of these classes evidently being due to the elimination of male gametes bearing the factor for narrow leaves and femaleness), 630 broad-leaved males, and 463 narrow-leaved males. In this cross all four types should have appeared in equal numbers.[1] The

[1] The effect of the linked factors "narrow leaves" and "femaleness" on the production of male and female gametes can be most readily seen by the use of this simple diagram

	♂ Fb	fb
♀ FB	Fb FB 2	fb FB 630
Fb	Fb Fb 0	fb Fb 463

in which the male gametes are placed on the upper side of the square and the female gametes on the left side, while the number of each of the types of progeny are placed within the small squares, with their respective gametic combinations.

inequality of the last two classes must be due to inequality in production of the two kinds of female gametes FB and Fb; the latter, which carries femaleness and narrow leaf linked, appearing less frequently than FB. Apparently with the suppression or loss of the determiner for broad leaves in the sex chromosome, there has also been a partial suppression of a factor necessary for the normal development of male and to a less extent of female gametes. In personal correspondence with Dr. Shull he informs me that there is actually a great deal of pollen sterility in the narrow-leaved Lychnis male.

CONCLUSIONS

(1) The flowers of Fragaria are pentamerous with regard to all parts except pistils. The stamens are arranged in three whorls; the outer parapetalous series of 10 stamens, the middle antipetalous, short filamented series of 5, and the inner antisepalous series of 5. Increases in stamen number are due to the addition of 5, or a multiple of it, to either the antipetalous or the antisepalous series. Decreases in stamen number are due to the loss of first the antipetalous and next the antisepalous series. Apparently the parapetalous series are permanent. Decrease in stamen number is in no way related to dieciousness.

(2) There is a positive correlation between flower position, flower part number, and size of fruit in the strawberry.

(3) The wild American species of strawberry, from which the cultivated varieties have been derived, are for the most part diecious. The pistillate plants bear staminodia, which rarely develop as far as the pollen mother cell stage, and the staminate plants bear pistils which superficially appear to be perfect but which are only occasionally functional. In a few wild clones of *F. virginiana*, which appear to be sterile, pollen development is carried as far as the tetrad division or slightly beyond this to the liberation of the microspores, when complete disintegration of the anther contents to an oily mass takes place. In other instances a portion of the microspores develop normally while the remainder within the same anther disintegrate, while in other clones shortly after liberation, and following a slight growth of the microspores, complete abortion of the same type as that found in hybrids takes place. These anther types, in wild clones, all appear to be various expressions of a tendency toward dieciousness and are not the result of hybridization. Similar anther types are common in certain cultivated varieties, on the early flowers of an inflorescence, and especially on those appearing early in the season.

(4) There is a correlation between flower position and fertility of pistils, fertility decreasing in the later flowers of an inflorescence. Pistil sterility is expressed in the production of irregularly shaped berries or entirely sterile flowers. Sterility of the later flowers of an inflorescence is more general in hermaphrodites than in pistillates, suggesting that the hermaphrodites have been derived from staminates of the diecious wild forms.

(5) The appearance of considerable amounts of aborted pollen in wild plants of *F. virginiana* and *F. americana* is rare except in anthers of the intermediate type. Most cultivated varieties produce considerable amounts of aborted pollen of the type common in hybrids. The percentage of aborted grains is not constant in the individual flowers of a variety, and neither is it constant in the individual anthers of a single flower, as just as great variations appear within the anthers of a flower as are shown by composite pollen samples of individual flowers.

(6) In those varieties producing high percentages of aborted grains a portion, at least, of the morphologically normal pollen grains are functional, as shown by germination and bagging tests. There is no evidence of a physiological self-sterility in the strawberry.

(7) In the partially sterile variety Minnesota 3 pollen development is carried on normally up to the liberation of the microspores from the tetrad. At this time all of the microspores appear normal and alike. Following liberation, variations in rate of growth, time of division of the microspore nucleus, and ability of the individual microspores to develop normally are shown. At all stages, during this growth period microspores were found in various stages of abortion. *F. virginiana* exhibits as great regularity during this growth period as is shown in the stages leading up to liberation of the microspores.

(8) Liberation of the microspores from the tetrad marks the beginning of an independent gametophytic generation, so far as the metabolic processes of growth are concerned. The individual microspores float in a homogeneous nourishing medium provided by the sporophyte, but the use of this food material in cell metabolism depends entirely upon the individual organization of the microspores.

(9) Specific chromosome combinations have been shown by various investigators to be a potent factor in the development or lack of development of individual plants or animals. In plants heterozygous for a number of factors, as are the varieties of strawberries, numerous new chromosome combinations occur for the first time in the microspores. The varying rates of growth, time of microspore division, ability to increase the cytoplasm, and inability in many cases to develop normally seem to be the outward expression of the differential ability of these new chromosome combinations to carry on cell metabolism.

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PLATE B

Minnesota 3:

1.—Pollen mother cell previous to synapsis. × 2,000.

2.—Presynapsis in the pollen mother cell showing loops extending out from synaptic mass. × 2,000.

3.—Two loops and portion of a loop extending from the presynaptic mass. Same stage as figure 2. × 3,280.

4.—Synapsis in a pollen mother cell. × 2,000.

5.—A postsynaptic stage. The synaptic mass is unraveling into bivalent loops. × 2,000.

6.—Open spireme stage. × 2,000.

7.—A presegmentation stage of the spireme. × 2,000.

8.—A portion of a bivalent spireme thread of the same stage as figure 7. × 3,280.

9.—Segmentation of the bivalent spireme into chromosome pairs. The cytoplasm is rounding up and is partially surrounded by a gelatin-like sheath. × 2,000.

10.—A portion of the bivalent spireme during segmentation. × 2,340.

11, 12.—Chromosome pairs during the contraction period following segmentation. × 2,000.

13.—Individual chromosome pairs showing various figures commonly formed during contraction. × 3,280.

14.—Diakenesis in the pollen mother cell. × 2,000.

15.—Multipolar spindle stage of pollen mother cell. × 2,000.

16.—Early anaphase of the heterotypic division. × 3,280.

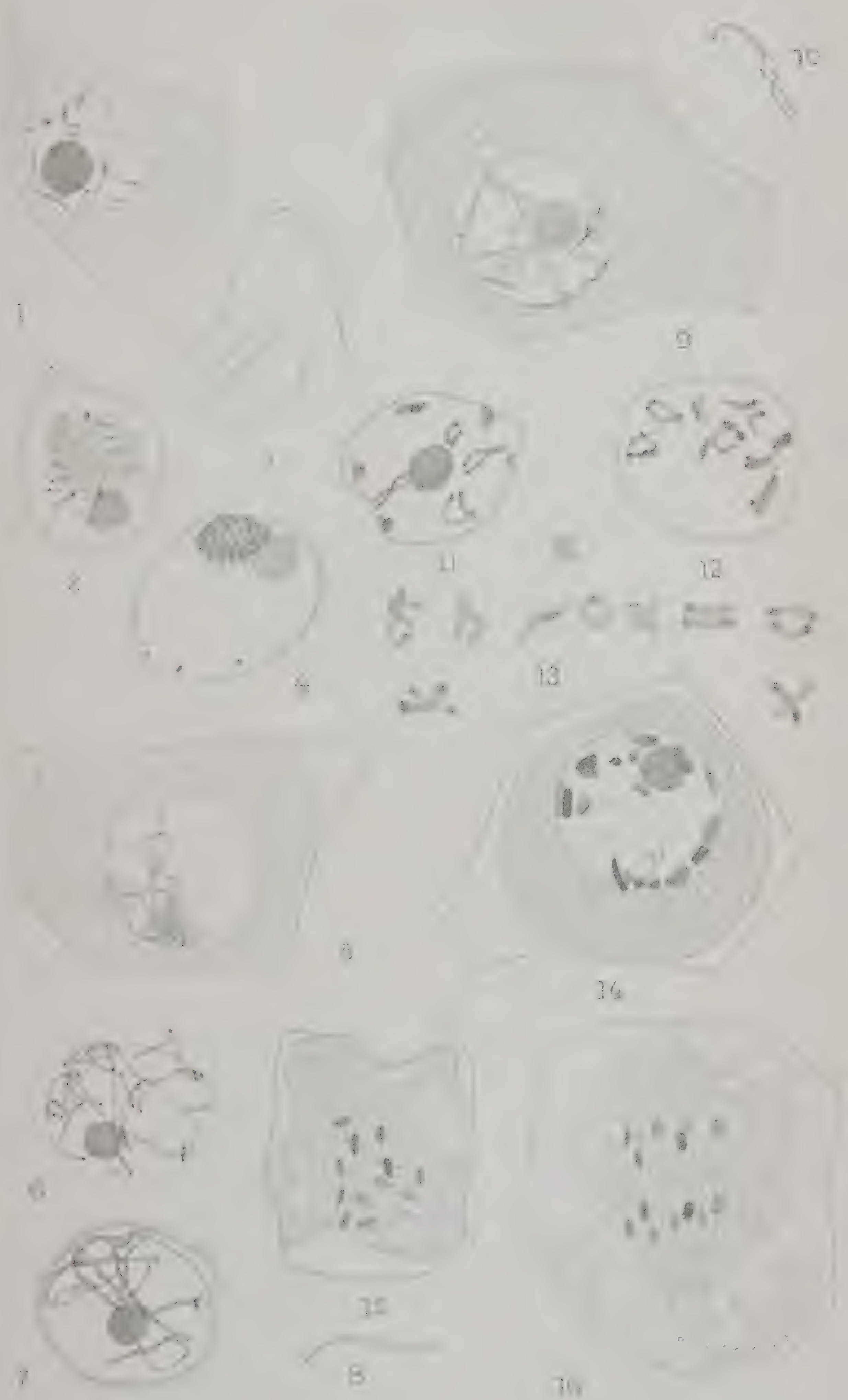

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PLATE C

1.—Late anaphase of the heterotypic division. The cytoplasm is rounding up from the mother cell wall and is partially surrounded by a thick gelatin-like sheath. × 2,000.

2.—Chromosomes on the equatorial plate of the homeotypic division.

3.—A portion of an anther in the tetrad stage, showing the microspores embedded in the gelatin-like sheath. The original mother cell walls are still present.

4.—A tetrad at the same stage as those shown in figure 3. The mother cell wall is not shown. × 2,000.

5.—A microspore shortly after liberation from the tetrad. × 2,000.

6.—A liberated microspore in which growth has commenced. × 2,000.

7.—A later stage than figure 6, showing the slight thickening of the wall and the irregularities due to growth of the wall. × 2,000.

8.—Microspore growth completed previous to division of the microspore nucleus. × 2,000.

9.—A section through a microspore nucleus in prophase showing the continuous univalent spireme. × 3,280.

10.—Another section of the same nucleus, showing the first stages of the disappearance of the nucleolus. The nucleolar strands are attached to the spireme. × 3,280.

11.—Metaphase of the division of the microspore nucleus. A spindle in this position results in the nuclear arrangement shown in figure 15. The thickened extine is shown. × 2,000.

12.—*F. virginiana*. Anaphase in the division of the microspore nucleus. The spindle lying parallel to the wall results in the nuclear arrangement shown in figure 14. × 2,000.

13.—Telophase of the division of the microspore nucleus. The wall which eventually surrounds the generative nucleus is not always apparent at this time.

14.—A later stage than figure 13 in which the generative cell has been definitely cut off. × 2,000.

15.—A young pollen grain shortly after division, showing an increase in cytoplasm content. The thickened extine is shown. × 2,000.

16.—End view of a pollen grain showing the pattern of the laminate layers shown in figure 15 and Plate D, figures 1, 6, and 15. The arrows mark the ends of the three sutures which bear the germ pores.

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PLATE D

1.—Nearly mature pollen grain. The central body is the vegetative nucleus while the other is the generative cell. × 2,000.

2.—Mature pollen grain. The extine is not shown. The killing fluid causes the dry folded grains to become spherical. × 2,000.

3, 4, 5, 7.—Various types of degenerate microspores from anthers bearing microspores of the stage shown in Plate C, figure 6. × 2,000.

6.—An aborting microspore from an anther containing half-grown microspores. × 2,000.

8.—An aborting microspore of the same type as that shown in figure 6 from an anther containing nearly full-grown microspores as in Plate C, figure 8. As in Plate D, figure 6, the cytoplasm and nucleus still appear normal. × 2,000.

9, 11.—Microspores of the same types and same age as figures 6 and 8, in which degeneration has proceeded farther. × 2,000.

10.—An aborted microspore from an anther containing microspores of the stage shown in Plate C, figure 8. Apparently abortion took place shortly following liberation from the tetrad.

12.—An early stage of degeneration in a full-grown 1-nucleate microspore. × 2,000.

13.—An early stage of degeneration in a full-grown 1-nucleate microspore. A number of normal microspores in this anther are already dividing.

14.—An aborting microspore containing an abnormally small amount of light staining cytoplasm; from an anther containing 1- and 2-nucleate microspores. × 2,000.

15.—An aborted microspore from an anther containing 1- and 2-nucleate microspores. Apparently this is a late stage of the type of degeneration shown in figures 6 and 8. × 2,000.

16.—An aborted microspore containing very scant cytoplasm. The nucleus has completely degenerated and degeneration of the cytoplasm has begun.

PLATE E

1.—A slightly more advanced stage of the condition shown in Plate D, figure 16.

2.—An early stage in the abortion of a full-grown 1-nucleate microspore. × 2,000.

3.—An early stage of abortion directly following microspore division. × 2,000.

4.—A full-grown 1-nucleate microspore containing very scant light-staining cytoplasm; from an anther containing 1- and 2-nucleate microspores. × 2,000.

5.—Another type of degeneration of a full-grown 1-nucleate microspore. × 2,000.

6.—An aborted microspore found among 1- and 2-nucleate microspores. × 2,000.

7.—A later stage of the type of degeneration shown in Plate D, figure 13; from an anther containing microspores of the stage of development shown by Plate C, figure 15. × 2,000.

8.—Degeneration of the generative cell shortly after division. The vegetative nucleus and cytoplasm are still normal. × 2,000.

9, 10.—Common types of aborted microspores found with mature pollen. Evidently abortion took place before the division of the microspore nucleus. × 2,000.

11.—An aborted microspore, of the same type as that shown in figure 7. Found with mature pollen. × 2,000.

12.—A pollen grain showing abortion of the generative cell and an abnormal vacuolate condition of the cytoplasm. × 2,000.

13.—A later stage of the type of degeneration shown in figure 8. The vegetative nucleus and cytoplasm are normal. × 2,000.

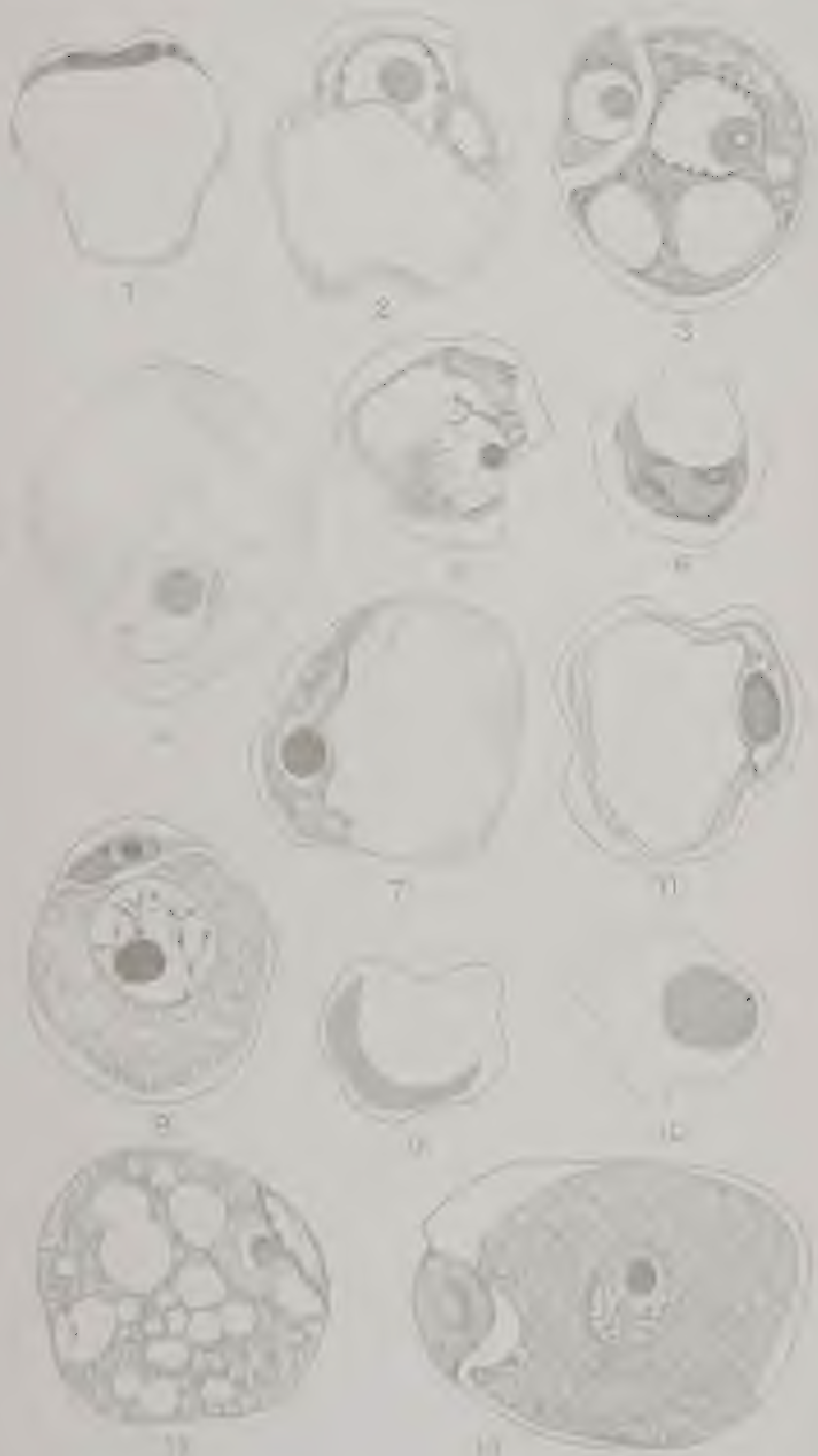

PLATE 35

A.—Tertiary flower of the pistillate variety, Minnesota 1017×Progressive–13–40, showing prominent staminodia.

B, C.—Primary and secondary flowers of the perfect variety, Minnesota 1017×Progressive–9–24; B showing intermediate and C perfect anthers.

D, E, F.—Two primary and a secondary flower of the perfect variety, Minnesota 1017×Progressive–2–55, showing pistillate, intermediate, and perfect types of flowers.

G, H, I, J.—Flowers from the perfect variety, Minnesota 1017×Progressive–32–1. G and H are primary and secondary flowers, respectively, and are pistillate; I a secondary imperfect flower with a few normally developed stamens and J a tertiary perfect flower.


A
B
C
D
E
F
G
H
I
J

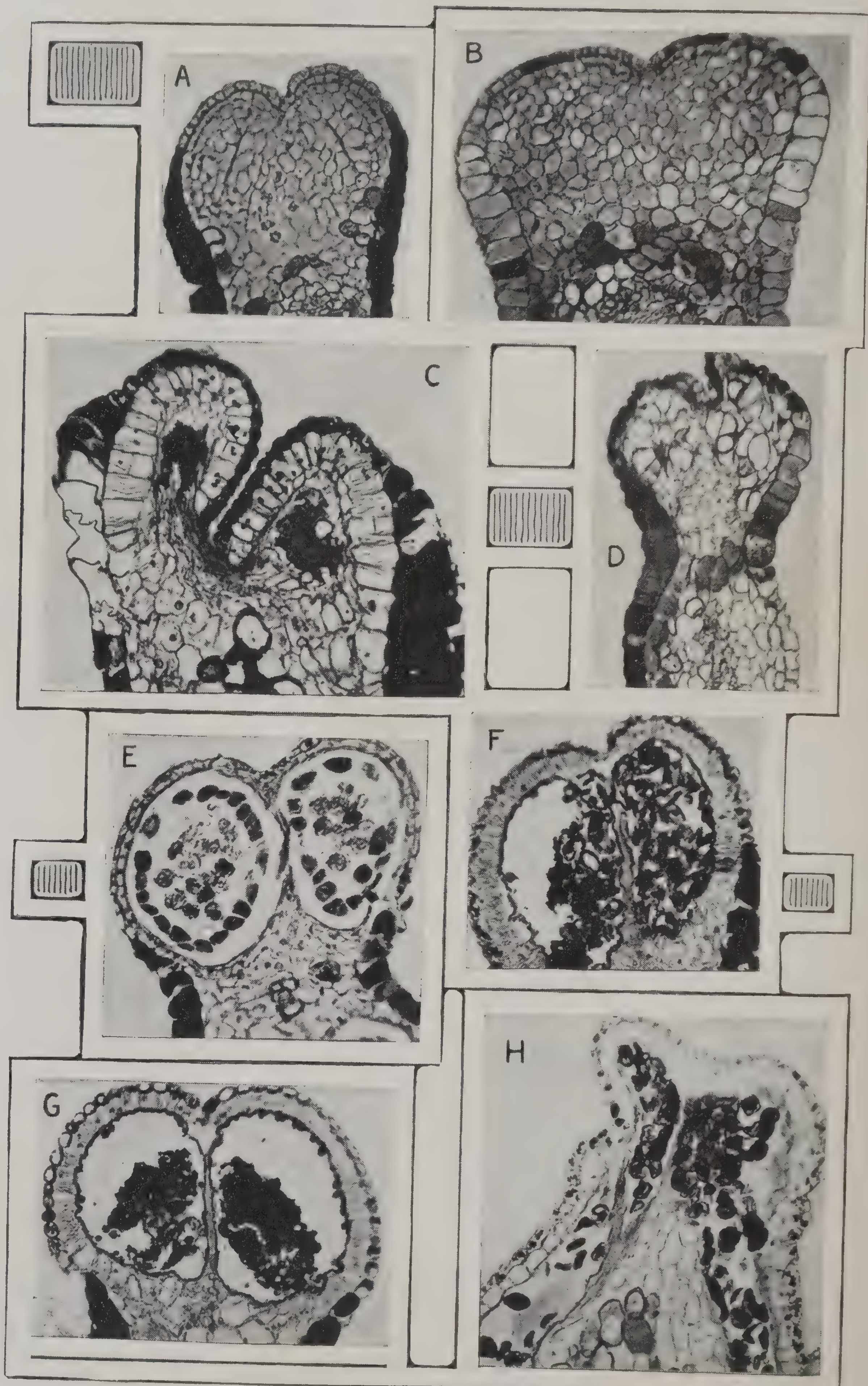
A
B
C
D
E
F
G
H

PLATE 36

A, B, C, D.—Cross sections of two loculi of staminodia of the pistillate varieties, Crescent, Columbia, Minnesota 1017×Progressive—11–59, and Seedling 140, respectively.

E.—Degeneration of the tetrads in an intermediate anther of *Fragaria virginiana*.

F, G.—Later stages of the condition shown in figure E.

H.—A portion of an intermediate anther from the first flower of Minnesota 3.

EFFECT OF NITRIFYING BACTERIA ON THE SOLUBILITY OF TRICALCIUM PHOSPHATE[1]

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INTRODUCTION

The solution of tricalcium phosphate and the chemical changes through which it passes in soils are subjects of special interest. The phosphorus compounds of soils have been mainly derived from tricalcium phosphate, and are relatively insoluble in water. A considerable part of the phosphorus of soils probably actually occurs as tricalcium phosphate. The soluble phosphates of processed fertilizer also become relatively insoluble soon after being mixed with soil, some of which may be converted into tricalcium phosphate. In addition, tricalcium phosphate, in the form of untreated rock phosphate, has been recommended as a fertilizer at various times in the past and at present is being applied in considerable amounts in different parts of America.

While tricalcium phosphate is relatively insoluble in water, it is well known that this substance is notably soluble in water saturated with carbon dioxid. For this reason it has long been suspected that the carbonic acid of soils promotes the solution of the phosphates present. Likewise the increased fertilizing effect resulting from the application of rock phosphate in conjunction with decaying organic matter has commonly been assumed to be due to the solvent effects of the carbonic acid, and possibly other organic acids, that are formed in the decomposition of the organic matter.

As a rule, however, investigators have not been able to detect any increase in the solubility of rock phosphate when left in contact with decaying organic matter. For example, Lupton (*6*)[3], McDowell (*7*), Truog (*9*), Tottingham and Hoffmann (*8*), and various European workers[4] found, as a result of composting rock phosphate with various fermenting mixtures, that in no case more than slight increases in the solubility of the phosphate took place and in certain instances decreases in solubility were noted.

[1] Paper No. 45, University of California Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, Cal.

[2] Credit is due Mr. A. B. Cummins for assistance in this investigation.

[3] Reference is made by number (italic) to "Literature cited," p. 683.

[4] A very complete bibliography of this subject is given by Lipman, McLean, and Lint (*5*).

A number of investigators have shown that the solution of tricalcium phosphate may be effected by the biochemical oxidation of sulphur. Recently Lipman, McLean, and Lint (5) found that large amounts of rock phosphate may be made soluble by the sulphur bacteria in fermenting mixtures containing elemental sulphur.

The effects of the nitrifying bacteria on the solubility of tricalcium phosphate have recently been investigated by Hopkins and Whiting (3). They found that the nitrite bacteria (*Nitrosomonas* spp.) have the power of oxidizing ammonium sulphate in solution cultures containing tricalcium phosphate but no carbonate or free base, and that under these conditions the nitrous acid and sulphuric acid, formed from the ammonium sulphate, attacked the tricalcium phosphate and rendered notable amounts of phosphorus and calcium soluble in water. Similar effects were found when ammonium nitrate was substituted for ammonium sulphate. By calculation they found, as an average of 13 tests, that for every 56 pounds of nitrogen oxidized, 115 pounds of phosphorus and 211 pounds of calcium were made soluble in water. They also found that no change in the solubility of tricalcium phosphate takes place as a result of the action of the nitrate bacteria (*Nitrobacter* spp.). As pointed out by them, the oxidation of nitrite to nitrate does not necessitate an increase in acidity, but is merely a matter of adding an atom of oxygen to the nitrite.

Hopkins and Whiting have discussed their views regarding the practical bearing of these experiments at considerable length, and have assigned special importance to the nitrite bacteria as agents in promoting the solution of rock phosphate in the field. In commenting on these investigations Davenport [1] even suggested that the importance of the nitrite bacteria as agents in the solution of rock phosphate, is on a par with that of the legume bacteria in nitrogen fixation.

Since the formation of nitrous acid is commonly considered to be an essential step in the nitrification process and as nitrification is generally active in productive soils, it is at once apparent that relatively large amounts of phosphate will be made soluble by this group of bacteria, provided the reactions that take place in soils be similar to those found in solution cultures.

The practical importance of phosphates in agriculture and the general interest in the several phases of the nitrification process justify further investigation of this problem. Accordingly, the writer has made some studies on it at the University of California Citrus Experiment Station. In these studies both soil and sand cultures have been employed. The formation of nitrate and the solubility of calcium and phosphoric acid in water were used in this investigation as measures of the biochemical action of the nitrifying organisms.

[1] Foreword to the publication by Hopkins and Whiting (3).

EXPERIMENTAL RESULTS

The soil used in this investigation was drawn from one of the plots (F) now being used in a fertilizer experiment with Citrus trees. This plot has been treated annually for 10 years with light applications of stable manure, but no commercial fertilizer or lime has been applied to it. The soil is a light sandy loam of granitic origin, the coarser particles of which are composed largely of granite. It contained 8.5 p. p. m. of nitric nitrogen, and 0.188 per cent of total phosphorus pentoxid (P_2O_5), of which 17.4 p. p. m. were soluble in water when the experiment was begun. The total carbonate (CO_3), as determined by the Gaither (*2*) method, amounted to 0.03 per cent, but the sample was free from water-soluble carbonate (CO_3).

Portions of 2,000 gm. each of fresh soil were weighed into half-gallon (1.89 liters) fruit jars. A solution of ammonium sulphate (c. p.) was added to certain portions at rates supplying 0.01 gm. of nitrogen per 100 gm. of dry soil. To other portions an equal quantity of nitrogen was added in the form of dried blood. Still other portions were employed without the addition of any nitrogenous substance. Baker's analyzed tricalcium phosphate was added in certain cases at the rate of 0.10 gm. per 100 gm. of soil and calcium carbonate (c. p.) at the rate of 0.25 gm. per 100 gm. of soil. The experiments were made in duplicate. After a thorough mixing, adding suitable amounts of water and mixing again, the jars were loosely covered and incubated at room temperature.

It is, of course, well known that the purest tricalcium phosphate is somewhat soluble in water and that ammonium sulphate affects the solubility of certain soil constituents, notably calcium, without the intervention of bacteria. Consequently it was deemed necessary to determine the solubility of calcium and phosphoric acid after the above-named substances had been mixed with the soil, but before sufficient time had elapsed to permit measurable bacterial action. It is obvious that the amounts of soluble calcium and phosphoric acid present in the soil at the beginning of the experiment should not be considered as having been dissolved by subsequent bacterial action. Accordingly portions of soil each containing 200 gm. were placed in flasks, the same relative proportions of tricalcium phosphate, calcium carbonate, and ammonium sulphate added as in the incubated series, and the contents thoroughly mixed. Distilled water was added at the rate of 250 parts per 100 parts of dry soil, and the contents were vigorously shaken once every 10 minutes during an hour, and were then filtered through Chamberland-Pasteur filters. Calcium was determined in the filtrates by the volumetric permanganate method and phosphoric acid by the Pemberton volumetric method. The average results obtained from closely agreeing duplicate solutions, expressed in parts per million of dry soil, are submitted in Table I.

TABLE I.—*Solubility of calcium and phosphate in soil immediately after adding calcium carbonate, tricalcium phosphate, and ammonium sulphate*

Treatment.	Soluble calcium (Ca).	Soluble phosphoric acid (P_2O_5).
	P. p. m.	*P. p. m.*
Soil only	27.5	17.4
Soil and calcium carbonate	26.8	13.8
Soil and tricalcium phosphate	31.1	28.6
Soil, tricalcium phosphate, and calcium carbonate	33.5	27.1
Soil and ammonium sulphate	90.1	16.6
Soil, ammonium sulphate, and tricalcium phosphate	92.6	25.1

These data show that the addition of calcium carbonate produced no effect on the immediate solubility of the calcium already in the soil or that added as tricalcium phosphate, but the addition of tricalcium phosphate produced an increase of about 5 p. p. m. of soluble calcium and 11.2 p. p. m. of soluble phosphoric acid. The most notable effect was produced by ammonium sulphate, which caused an increase in water-soluble calcium from 27.5 to 90.1 p. p. m.

The data submitted in Table I should not be considered as representing true solubility determinations, for it is not certain that equilibrium was completely established, either between the various solids present and the solvent (water), or between the constituents of the soil and the chemical substances that were added to it. A longer period of contact might have yielded extracts either more or less concentrated, depending on whether or not the rate of solution was greater or less than the rate of fixation. The same procedure was followed in making these determinations, however, as was used at the close of the incubation periods, and, although the results are not strict solubility determinations, they are believed to be comparable, and that any difference between the amounts of calcium and phosphoric acid found at the beginning and the close of the periods of incubation may be assumed to have arisen mainly through the action of biochemical agents.[1]

After incubation periods of 28, 57, and 157 days, quantities containing 200 gm. of dry soil were transferred from the incubation jars to flasks, distilled water was added at the rate of 250 parts per 100 parts of dry soil, was shaken vigorously once every 10 minutes during an hour, and was then filtered through Chamberland-Pasteur filters as in the preceding series. Soluble calcium and phosphoric acid were determined in the filtrates by the methods already referred to, and nitric nitrogen by the phenoldisulphonic-acid method. The filtrates were also tested for nitrite, but not more than 0.5 p. p. m. was found in any case. The average results of closely agreeing duplicates are recorded in Table II.

[1] It was not deemed advisable to maintain separate portions in a sterile condition, owing to the fact that the various methods now in use for bringing about complete sterilization in soils probably affect the solubility of the various constituents.

TABLE II.—*Effects of nitrification on the solubility of tricalcium phosphate in soil*

Materials added.	After 28 days.			After 57 days.			After 157 days.		
	Nitric nitrogen.	Calcium.	Phosphoric acid.	Nitric nitrogen.	Calcium.	Phosphoric acid.	Nitric nitrogen.	Calcium.	Phosphoric acid.
	P. p. m.	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*
Control	20.0	45.0	13.1	25.5	50.6	11.0	......	......	......
Calcium carbonate	22.0	56.5	11.9	29.0	70.8	13.2	......	......	......
Tricalcium phosphate	21.0	53.5	24.2	28.0	58.8	25.0	......	......	......
Calcium carbonate and tricalcium phosphate	22.0	59.1	17.3	28.0	70.1	22.4	......	......	......
Ammonium sulphate	98.0	219.4	18.5	99.0	225.4	19.4	114.0	232.1	8.0
Ammonium sulphate and calcium carbonate	97.0	254.4	18.5	98.0	270.5	7.4	......	......	......
Ammonium sulphate and tricalcium phosphate	99.0	217.7	52.1	99.0	229.6	38.0	111.0	218.4	30.0
Ammonium sulphate, calcium carbonate, and tricalcium phosphate	100.0	253.4	26.6	101.0	230.4	13.9	......	......	......
Dried blood	91.0	107.7	9.7	90.0	113.9	10.0	94.0	116.4	5.7
Dried blood and calcium carbonate	89.0	107.2	9.8	90.0	140.2	11.5	......	......	......
Dried blood and tricalcium phosphate	82.0	111.7	24.3	88.0	117.7	22.2	......	......	......
Dried, blood calcium carbonate, and tricalcium phosphate	81.0	118.2	19.5	87.5	138.1	18.3	......	......	......

A series of experiments with the use of silica sand corresponding closely with the preceding soil series was conducted at the same time. The silica sand was obtained from Monterey, Cal., and was free from carbonate, but contained small amounts of feldspar, hornblende, and mica particles, and possibly traces of other minerals. Portions of 1,000 gm. each were placed in fruit jars, and quantities of ammonium sulphate, dried blood, calcium carbonate, and tricalcium phosphate were added in duplicate at the same rates and arranged after the same plan as in the preceding soil series.

Mixed cultures of bacteria were supplied by adding 150 c. c. of an ordinary soil infusion obtained from the soil used in the preceding series. The infusions were quite clouded with suspended matter, which probably included small amounts of various soil constituents. In addition, 50 c. c. of a nutrient solution, composed of 2 gm. of sodium chlorid, 0.2 gm. of magnesium sulphate, 0.5 gm. of potassium sulphate, and 6 drops of a 10 per cent solution of ferric chlorid per liter, were thoroughly mixed with the sand in each jar. The jars were loosely covered and incubated at room temperature.

After periods of 28, 56, 98, and 157 days, quantities containing 200 gm. of dry sand were withdrawn, 500 c. c. of distilled water added, and after shaking vigorously as in the preceding series, were filtered through

Chamberland-Pasteur filters. Nitrate, calcium, and phosphoric acid were determined in the filtrates by the methods already referred to. Nitrite, when present, was also determined by the Greiss-Ilosovay method. The calcium determinations for the 98-day period have been omitted from the table, owing to an error in the analytical procedure. The average results from duplicate incubations are recorded in Table III.

TABLE III.—*Effects of nitrification on the solubility of tricalcium phosphate in sand cultures*

[Results expressed in parts per million]

Materials added.	After 28 days.				After 56 days.				After 98 days.			After 157 days.			
	Nitric nitrogen.	Nitrous nitrogen.	Calcium.	Phosphoric acid.	Nitric nitrogen.	Nitrous nitrogen.	Calcium.	Phosphoric acid.	Nitric nitrogen.	Nitrous nitrogen.	Phosphoric acid.	Nitric nitrogen.	Nitrous nitrogen.	Calcium.	Phosphoric acid.
Calcium carbonate	7.5	0.0	72.5	0.0	9.0	0.0	77.6	0.0	12.2	0.0	0.0	13.0	0.0	77.6	0.0
Tricalcium phosphate	7.2	.0	52.1	67.5	8.1	.0	51.8	52.3	10.0	.0	53.3	10.2	.0	44.6	45.7
Calcium carbonate and tricalcium phosphate	7.3	.0	75.1	23.7	8.3	.0	61.3	18.0	11.5	.0	21.6	10.5	.0	64.3	19.8
Ammonium sulphate	1.3	.0	26.0	.0	1.1	.0	24.5	.0	1.5	.0	.0	6.9	.0	28.7	.0
Ammonium sulphate and calcium carbonate	92.0	.0	308.0	.0	79.0	.0	298.0	.0	82.5	.0	.0	92.0	.0	345.1	.0
Ammonium sulphate and tricalcium phosphate	2.1	.0	62.6	77.9	7.5	.0	87.1	79.9	14.0	.0	83.9	33.0	.0	120.4	85.1
Ammonium sulphate, calcium carbonate, and tricalcium phosphate	33.5	8.2	207.8	17.7	80.0	.0	269.2	12.5	80.0	.0	15.4	92.0	.0	304.0	15.8
Dried blood	1.1	41.2	23.0	.0	3.0	30.0	20.5	.0	7.2	23.7	.0	29.0	5.0	35.5	.0
Dried blood and calcium carbonate	45.0	15.6	150.3	.0	75.0	.0	191.5	.0	81.0	.0	.0	91.0	.0	210.6	.0
Dried blood and tricalcium phosphate	.5	25.0	38.5	57.1	1.0	31.0	40.3	55.7	2.0	25.0	52.2	12.2	22.5	45.5	46.5
Dried blood, calcium carbonate, and tricalcium phosphate	1.3	62.5	100.2	15.1	2.9	52.0	115.7	12.0	2.6	50.0	18.3	11.0	75.0	162.0	12.7

DISCUSSION OF EXPERIMENTAL RESULTS

SOIL SERIES.—The data submitted in Table II show that active nitrification took place in the soil series. For example, the nitric nitrogen increased in the control portions from 8.5 p. p. m., originally present, to 20 p. p. m. in 28 days. Where ammonium sulphate was added, the concentration increased to 98.5 p. p. m., while dried blood yielded 91.0 p. p. m. of nitric nitrogen. The addition of calcium carbonate and tricalcium phosphate either singly or together produced very slight, if any, effects on nitrification in this series.[1] After subtracting the amounts of nitric nitrogen in the controls, it is found that 78 per cent of the ammonium sulphate and 71 per cent of the dried-blood nitrogen were oxidized in 28 days.

When allowance is made for the soluble calcium found at the beginning of the experiment (Table I), the data show that in every case nitrification

[1] In other experiments with this soil the addition of calcium carbonate has slightly stimulated the nitrification of ammonium sulphate, but not of dried blood.

was accompanied by increases in the solubility of the calcium present.[1] This was noted to some extent in the portions to which no nitrogenous additions were made; was considerably greater when dried blood was supplied; and was greatest with the addition of ammonium sulphate. The data show, however, that the concentration of soluble calcium was not increased as a result of adding tricalcium phosphate. On the other hand, soluble calcium was considerably increased in a number of cases by the addition of calcium carbonate. It would seem, therefore, that calcium, in the form either of the carbonate or of such silicates as occur in this soil, will be dissolved by the biochemical oxidation products in preference to tricalcium phosphate.

In contrast to the effects on the solubility of calcium, the data show that nitrification of the soil nitrogen and that added as dried blood was accompanied in each case by a well-defined decrease in soluble phosphoric acid. Where ammonium sulphate was added alone, the amounts of soluble phosphoric acid found at the 28- and 57-day periods were approximately the same as found at the beginning of the experiment. It would appear, therefore, that the solvent action of the bacteria in this case was almost exactly equal to the precipitating action that evidently took place in the control and dried-blood portions. After 157 days, however, more than half of the soluble phosphoric acid originally present in this portion had disappeared. It is also shown that, while larger amounts of soluble phosphoric acid were found where tricalcium phosphate had been added than in the control portions, the increases can not be definitely ascribed to the action of bacteria in any case except where ammonium sulphate was also added, and then only without the addition of calcium carbonate. For in all other cases the solubility immediately after adding tricalcium phosphate was equal to or greater than that at the end of the incubation periods.

In the absence of calcium carbonate, however, the oxidation of ammonium sulphate dissolved tricalcium phosphate, as shown by the fact that the concentration of soluble phosphoric acid was increased in 28 days from 24.2 to 52.1 p. p. m. Later the solubility steadily declined until at the end of 157 days the concentration had been reduced to 30 p. p. m.

Assuming in this case that the increase in nitric nitrogen over the amounts found in the controls was due to the oxidation of ammonium sulphate, we find that the oxidation of 78.0 p. p. m. of nitrogen resulted

[1] It should be clearly understood that the results obtained in this investigation represent the algebraic sum and difference of the results of a number of forces. In the first place it is highly probable that biochemical agents other than the nitrifying organisms are capable of affecting the solubility of calcium and phosphoric acid in soils. A part of this effect may be referred to as positive and a part as negative, since, on the one hand, carbonic acid, formed in the life process of bacteria, is a solvent for calcium and phosphoric acid, and on the other hand, the organisms themselves absorb phosphoric acid (8). In the second place diffusion tends to bring about more or less fixation in soils. The concentration at a given moment, therefore, is really dependent on the interaction of a number of forces. Consequently a full explanation of the results obtained is not possible at present.

in the solution of 27.9 p. p. m. of phosphoric acid, or 12.2 p. p. m. phosphorus. By comparing these data with the rate of solution reported by Hopkins and Whiting from solution cultures, it will be seen that, while the oxidation of 1 pound (454 gm.) of nitrogen was accompanied by the solution of 2.033 pounds (922 gm.) of phosphorus in their experiments;[1] in these experiments with soil cultures the maximum amount of phosphorus made soluble was only 0.156 pound (70.8 gm.) per pound of nitrogen oxidized. Therefore, the oxidation products of ammonium sulphate were approximately 13 times as effective in dissolving tricalcium phosphate in solution cultures as in this soil.

It is interesting to note that the addition of calcium carbonate tended to lower the solubility of tricalcium phosphate wherever applied.

SAND SERIES.—Ammonium sulphate, when added alone, underwent almost no nitrification in the sand series (Table III) until the last period of the experiment, during which small amounts of nitrate were formed.[2] The presence of calcium carbonate, however, promoted very active nitrification of ammonium sulphate. In this case the concentration of nitric nitrogen reached its maximum (92 p. p. m.) in 28 days. On the other hand, the effects resulting from the addition of tricalcium phosphate only began to be manifested by the fifty-sixth day. Later the nitrate content slowly increased until the close of the experiment, when 33 p. p. m. were found. Nitrification of ammonium sulphate in the proportions containing both calcium carbonate and tricalcium phosphate was not so pronounced during the first 28 days as with calcium carbonate only, but later the effects were almost identical.

The nitrification of dried blood in sand proved to be especially interesting in that the intermediate formation of nitrite proceeded much more rapidly than the oxidation to nitrate.[3] When dried blood alone was added, 42.1 p. p. m. of nitrite nitrogen were found after 28 days and only 1.1 p. p. m. of nitric nitrogen. Later no further accumulation of nitrite took place, but the formation of nitrate set in slowly with the result that 29 p. p. m. had been formed by the close of the experiment, but even then 5 p. p. m. of nitrite still remained.

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[1] It should not be inferred that Hopkins and Whiting claim that the products of nitrification will dissolve rock phosphate at the same rate in soil as in solution cultures. They pointed out (*3. p. 405*), for example, that nitrous acid may combine with calcium silicate, calcium carbonate, and other compounds in soils as well as with tricalcium phosphate. Nevertheless they hold that the nitrite bacteria are important agents in bringing about the solution of rock phosphate in field soils.

[2] Appreciable amounts of nitrate were formed in the portions which were intended to be free from combined nitrogen. The nitrate in these instances probably originated from organic matter held in suspension in the soil infusions that were added. The amounts formed, however, were small and consistent in every case, increasing steadily from an average of 7.3 p. p. m. at the 28-day period to a maximum of 11.2 p. p. m. at 98 days.

[3] Data showing that nitrites may accumulate in nitrification experiments have previously been reported (*4*), but this is a condition not commonly met in the field. The accumulation of nitrites indicates, of course, that some factor in the medium was abnormal, but more favorable for the nitrite bacteria than for the nitrate bacteria. It is known that the nitrate bacteria are more sensitive to adverse conditions than the nitrite bacteria.

The application of calcium carbonate notably stimulated the nitrification of dried blood, and after 56 days the yield was approximately the same as from ammonium sulphate. On the other hand, tricalcium phosphate produced no stimulation in the nitrification of dried blood at any period, but the application of both calcium carbonate and tricalcium phosphate promoted active nitrite formation, which during the last period of the experiment resulted in the oxidation of approximately as much nitrogen as in any other case in the experiment. The final oxidation to nitrate in this case, however, was very feeble throughout the entire experimental period.

It is evident from the above results, therefore, that, while tricalcium phosphate may promote nitrification in the absence of carbonate, more favorable conditions for nitrification were produced by calcium carbonate than by tricalcium phosphate.

Large amounts of calcium carbonate were made soluble by the nitrification of ammonium sulphate in the sand series, but since enfeebled nitrification of ammonium sulphate took place when tricalcium phosphate only was added, relatively small increases in soluble calcium were produced. At the final period of the experiment, however, the increase in soluble calcium resulting from tricalcium phosphate and the small amounts of nitrogen that had been oxidized agree closely with theoretical calculation.

Again, considerably less calcium was dissolved in the nitrification of dried blood than in that of ammonium sulphate, a result which is in harmony with generally accepted views regarding the nature of the oxidation products formed in the two cases. With the latter sulphuric acid is formed in addition to nitrous acid, while with the former carbonic acid is probably one of the end products.

The limited nitrification of dried blood, found where tricalcium phosphate had been added, was associated with a lower soluble-calcium content than occurred where tricalcium was added alone. But in view of the fact that not more than 30 per cent of the nitrogen was oxidized, it is probable that the medium remained alkaline as a result of the ammonification of the dried blood, and, consequently, the lower yields of soluble calcium may have been due to slight precipitation of calcium as calcium carbonate.

It is especially interesting that smaller amounts of calcium were dissolved by the nitrification of ammonium sulphate and dried blood in the presence of both calcium carbonate and tricalcium phosphate than with calcium carbonate alone.

Considering the phosphoric-acid determinations, it is at once apparent that nitrification did not produce an increase in the solubility of tricalcium phosphate when carbonate was present. In the absence of carbonate, however, an increase in solubility resulted from the nitrification of ammonium sulphate. In this case, although nitrification was

less active than elsewhere, the data show that the small amounts of oxidation products formed dissolved tricalcium phosphate. On the other hand, wherever calcium carbonate was also present, the oxidation products not only combined with it, but the initial solubility of the tricalcium phosphate was very materially lowered as well. This was true in the nitrification of both ammonium sulphate and dried blood.[1]

INTERPRETATION OF RESULTS

In making a practical interpretation of these investigations it should be borne in mind that fertile soils commonly contain at least small amounts of carbonate, that even the so-called acid soils frequently contain considerable amounts of bicarbonate, and that the presence of calcium carbonate in soils is generally considered to promote conditions that are favorable for the growth of most crops. Furthermore, large amounts of calcium carbonate are being applied to soils in many localities, especially in the humid sections. In the Central West, for example, ground limestone is being applied on a large scale, and generally it is recommended that the application be repeated every few years.

Under the conditions that result, chemical reasoning (*1*) and the experimental results reported above agree in suggesting that the action of the biochemical oxidation products, formed in the nitrification of organic nitrogen, would be spent on the carbonate and not on tricalcium phosphate. Furthermore, it seems doubtful whether this could be avoided by the application of limestone and rock phosphate at different times in a rotation, as was suggested by Hopkins and Whiting. Although it is possible that under this condition the particles of rock phosphate may chance to occur in local centers that are somewhat removed from solid particles of calcium carbonate, and nitrification may happen to take place in these centers, it does not even then necessarily follow that the phosphate would be dissolved. For such centers would probably always be in contact with soil silicates, and the above data indicate that at least some soil silicates may be attacked by the products of nitrification in preference to tricalcium phosphate. When the conditions permit the accumulation of considerable acidity, however, such as probably obtain when ammonium sulphate is applied to a soil low in carbonate, it was found that small amounts of the phosphate were dissolved. But then a degree of acidity that is distinctly injurious to crops may soon develop, as has been found by field trials in a number of localities. Furthermore, bicarbonate, arising from the action of carbonic acid on the solid particles of calcium carbonate, would certainly tend to diffuse toward the supposed centers of acidity, thus precipitating

[1] Soluble phosphoric acid may have been utilized to some extent in the life processes of the bacteria present, as was found by Tottingham and Hoffmann (*8*). But the simultaneous losses in soluble calcium also suggest the precipitation of phosphoric acid, a view that is in harmony with the well-known fact that calcium carbonate may precipitate phosphoric acid from solution.

the phosphoric acid. The results of many studies on the concentration of nitric nitrogen in soils supporting growing crops show, for example that diffusion is an important force in maintaining equilibrium in soils.

Tricalcium phosphate can not be converted into monocalcium phosphate (the water-soluble form) without active acidity being developed.[1] Nor are acids neutralized by converting tricalcium phosphate into monocalcium phosphate, since the latter is an acid compound. But when the acidity necessary to the solution of tricalcium phosphate is neutralized, the phosphoric acid will be precipitated. If it be desirable that a condition either of neutrality or slight alkalinity obtain in soils, as has been widely taught, it is difficult to see how more than traces of monocalcium phosphate can exist therein at the same time.

It should also be recalled that untreated rock phosphate generally contains considerable amounts of calcium carbonate intimately commingled with the phosphate. Before dilute acids, formed by the action of bacteria or otherwise, can dissolve the phosphate, the carbonate must first be neutralized, as is commonly recognized in the commercial processes employed in the manufacture of acid phosphate.

It seems possible, however, that the nitrifying bacteria may dissolve limited amounts of rock phosphate in acid soils. The soil used in this investigation, although low in carbonate, was not acidic, and more active solution of tricalcium phosphate would probably take place in an acid soil. But, in any case, with the possible exception of very sandy types of soil, it is probable that phosphoric acid, made soluble by the nitrifying bacteria, would tend to become fixed through being brought into contact with other soil constituents by diffusion. Many investigations have shown, for example, that acid phosphate soon becomes fixed, even in acid soils.

From these investigations it seems, therefore, that while the nitrite bacteria are capable of effecting the solution of tricalcium phosphate under restricted conditions, they are not the potent agents in the solution of rock phosphate in the field that Hopkins and Whiting were led to infer from their experiments with solution cultures.

In the opinion of the author the results obtained in this investigation should not be interpreted as being definitely opposed to the use of untreated rock phosphate as a fertilizer. It is true that the means by which tricalcium phosphate is made soluble in soil have not been definitely determined, but the important fact remains that in various localities beneficial effects on the growth of crops have frequently been produced by rock phosphate. It therefore remains for further investigation to lay bare the reasons. The suggestions offered by Truog (9) in this connection seem to be deserving of special consideration.

[1] It is, of course, understood that hydrolysis is excepted in this case. The absolute increases in soluble phosphoric acid in soils resulting from the hydrolysis of tricalcium phosphate are probably quite small.

SUMMARY

The investigations reported in this paper include a study of (1) the effects of adding calcium carbonate, tricalcium phosphate, and ammonium sulphate on the immediate solubility of calcium and phosphoric acid in a light sandy loam soil; (2) the effects of nitrification of the soil nitrogen, ammonium sulphate, and dried blood on the solubility of the naturally occurring calcium and phosphoric acid; (3) the effects of nitrification in soil and sand cultures on the solubility of tricalcium phosphate both with and without the application of calcium carbonate.

The following results were obtained:

(1) The addition of calcium carbonate produced no effect on the immediate solubility of the soil calcium or that added as tricalcium phosphate. The addition of tricalcium phosphate produced an increase of about 5 p. p. m. of soluble calcium and 11.2 p. p. m. of soluble phosphoric acid, while the addition of ammonium sulphate brought about an increase in water-soluble calcium from 27.5 to 90.1 p. p. m.

(2) Active nitrification of ammonium sulphate and dried blood took place in the soil series, and at the same time notable increases in soluble calcium were produced.

(3) No increase in the solubility in water of the soil phosphates or of tricalcium phosphate was produced by bacterial action except in the nitrification of ammonium sulphate when added without calcium carbonate. In this case 0.156 pounds (70.8 gm.) of phosphorous were dissolved for every pound of nitrogen oxidized, whereas Hopkins and Whiting found from solution cultures that 2.033 pounds (922 gm.) were dissolved for every pound of nitrogen oxidized.

(4) The addition of calcium carbonate brought about an increase in soluble calcium but tended to lower the solubility of tricalcium phosphate.

(5) In the absence of calcium carbonate the nitrification of ammonium sulphate in sand cultures was accompanied by the solution of theoretical amounts of tricalcium phosphate. When calcium carbonate was present, however, the solubility of tricalcium phosphate was not increased by nitrification.

(6) The formation of nitrite from dried blood took place more rapidly in the sand cultures than the formation of nitrate.

(7) Tricalcium phosphate was not dissolved by the nitrification of dried blood in the sand series.

(8) It was found that calcium carbonate promoted more active nitrification than tricalcium phosphate.

(9) The experimental results indicate that the nitrification of organic forms of nitrogen does not increase the solubility of rock phosphate under field conditions that are favorable to crop growth. It is possible, however, that the nitrification of ammonium sulphate may result in the solution of small amounts of tricalcium phosphate in soil low in carbonate.

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RESPIRATION OF STORED WHEAT[1]

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INTRODUCTION

The preservation in storage of large quantities of thrashed grain involves certain difficulties. In addition to protection from vermin, it has long been known that the grain must be dry when stored, and the ingress of moisture prevented. The ancients were aware that damp grain, or similar vegetable material, will heat and become decomposed when stored in quantities. It is evident that in prehistoric times carefully constructed receptacles were employed for the protection of the reserve supplies of cereals. Those ancient people of India, the Hindus, sometimes resort to the use of receptacles which are submerged in cool water, reducing the temperature of the stored grain and also the supply of air. In modern practice carefully constructed tanks or silos, frequently of large capacity, are chiefly used for the storage of grain.

CAUSE OF THE HEATING OF GRAIN

The cause of heating of damp vegetable matter was not known, however, until comparatively recent times, and it is only within the last decade or two that any data have been accumulated which indicate the exact effect of various factors on the rate of heating of grain and similar material. That the phenomenon known as respiration is responsible for the heat energy released in a mass of damp grain is shown by modern research. Loew (*1899*)[2], in discussing the fermentation and heating of leaf tobacco, maintained that the release of energy and rise in temperature was occasioned by the activity of the oxidizing enzyms of the leaf cells. The microbial flora were not believed to play any considerable part in these changes. In advancing this view he opposed vigorously the bacterial-fermentation theory of Suchsland (*1891*).

Rahn (*1910*) states that the curve of the process of spontaneous heating of organic matter, including grain, would not in itself indicate whether

[1] Published with the approval of the Director, as Paper 97, Journal Series, Minnesota Agricultural Experiment Station.

[2] Bibliographic citations in parentheses (italic) refer to "Literature cited," pp. 710–713.

the heat was produced by chemical or microbial causes. Gore (*1911, p. 33*) concluded that in self-heating, physiological processes are probably the first to operate. Oxidizing enzyms are the active agents in many cases. Chemical oxidation would intervene only when the temperature had been raised to the combustion point of the substances present. Gore presents formulas and graphs illustrating the theoretical progress of the accumulation of heat.

The studies of E. M. Bailey (*1912*) on the ripening of bananas indicate that bacterial activity is not responsible for the heat produced or other changes resulting during the ripening of the fruit.

Nabokich (*1903*) found that seeds which had been sterilized by chemicals such as mercuric chlorid respired during the first few days as much carbon dioxid as did the controls. In some instances the respiration of the sterilized seeds exceeds that of the controls.

Respiration may be briefly defined as the release of energy through the biochemical oxidation of organic compounds as accelerated by certain enzyms. Carbon dioxid and water are the characteristic chemical end products. It is shown by De Saussure (*1804*) that respiration was accompanied by the disappearance of oxygen. Pflüger (*1875*) maintained that the inspired oxygen combines in some manner with cyanogen radicals of the living protoplasm. This effects a readjustment, as the result of which carbon dioxid and water are eliminated. The decomposition is of an explosive character, and the reaction liberates heat energy.

Verworn (*1899, p. 483*) evolved the "biogen" theory, according to which the oxygen enters the "biogen molecules," which are thus rendered less stable. Slight impulses are then required to bring about a chemical union of this oxygen with the carbon in the cyanogen group.

Since the grain itself is a poor conductor of heat, it follows that the heat energy released through respiration accumulates in the mass in proportion to its bulk so that the increase in temperature may in time become very marked.

MATERIAL OXIDIZED IN RESPIRATION

In the case of resting tissues and storage structures, such as the grain kernel or caryopsis, the exact character of the substrate or material oxidized in respiration is of significance. Wehmer (*1892*) fed Aspergillus on peptone, and found it capable of satisfying its requirements from this source. Gore (*1914*) found that in ripening bananas the rate of starch hydrolysis paralleled the rate of respiration. There was slight change in the quantity of protein and fats in the fruit. Maige and Nicolas (*1910*) found that the immersion of etiolated leaves, shoots, or seedlings in sugar solutions resulted in an increased rate of respiration.

Langworthy and Milner (*1913*) observed that in ripening bananas the "thermal quotient," or quantity of heat produced per unit of carbon dioxid respired, indicated the combustion of carbohydrate. The thermal

quotient found for one period of their studies, when conditions for accurate measurement were at an optimum, was 2.6. This is so nearly the theoretical for the combustion of carbohydrates as to indicate that little else was involved. As pointed out by Dr. Milner in a private communication, the thermal quotient when fat is burned is 3.4, while that when protein is burned is 2.9.

Hasselbring and Hawkins (*1915*) found no general correlation between the total sugar content of the sweet potato and its respiratory activity. Cane sugar is relatively stable and does not appear to be used in the process of respiration. A simultaneous decrease in the reducing sugars and the respiratory activity was observed. The reducing sugars are, in their opinion, the immediate source of respiration material.

That the fat or ether extract of the wheat embryo is not the principal substrate upon which the respiratory enzyms act, and that it is not burned or destroyed during germination is indicated by the experiments of Le Clerc and Breazeale (*1911, p. 12*). Their data show that there was more fat in the total plant (seed residues plus axes) at practically all stages of germination than there was in the original seeds. Accordingly little energy could have been derived from the oxidation of the fats; on the contrary, energy derived from some other source must have been utilized in the synthesis of the additional fat produced in the seedling. All available evidence therefore seems to indicate that the heat of respiration is produced by the oxidation of reducing sugars.

SEAT OF RESPIRATION IN THE WHEAT KERNEL

There are a number of reasons for believing that the germ or embryo of the wheat kernel is the location of the larger part of the biological oxidation that occurs incidental to respiration. The embryo is, in a general way, decidedly richer in enzyms than is the endosperm or any other kernel structure. Brown and Morris (*1890*) consider that the endosperm of the ripened kernel is no longer a vital tissue. The secretion of diastase and other enzyms is assigned to the scutellum, an organ of the embryo. Mann and Harlan (*1915*) concluded that in germinating barley the conversion of the endosperm is effected by enzyms secreted by the epithelial layer of the scutellum.

Karchevski (*1903*) found the energy of carbon-dioxid respiration to be 12 times as great in the wheat embryos as in the seeds themselves. Burlakow (*1898*) states that the respiratory activity of the germ is 20 times greater than is that of the endosperm. Wender (*1905*) called attention to the pronounced catalase activity of the germ structures, as contrasted with mill products derived almost exclusively from the endosperm. This has also been observed in an unpublished study of catalases which was made in this laboratory. This fact is more significant, in view of Appleman's (*1915*) discovery that catalase activity in potato juice shows a striking correlation with respiratory activity in the

tubers. If catalase activity parallels respiratory activity, it may be reasoned by analogy that those structures of the wheat kernel which exhibit the greatest catalase activity are the seat of the larger part of respiration.

Barnes and Grove (*1916*) lend further support to the hypothesis that the seat of oxidation activity is in the embryo by their observation that in air-dry wheat the embryo becomes shrunken after a time, while the food materials of the endosperm are unimpaired. This is interpreted by them to indicate a destruction of the material of the embryo itself as the result of respiration when the dryness of the kernel suppresses diffusion. Were respiration equally pronounced in the endosperm it too should exhibit a similar loss of material.

Osterhout's (*1917*) observation that oxidation is more rapid in the nucleus than in the cytoplasm might lead to the deduction that, since the embryo cells of the wheat caryopsis have a much larger proportion of nuclei than the endosperm cells, oxidation should proceed more rapidly in the embryo tissues.

All of the above facts are in harmony with what might logically be expected. The principal release of energy as the result of biological combustion should occur in the structure where such energy is required for the synthesis of new organic compounds. Since the embryo is endowed particularly with that function, respiration must of necessity be most pronounced in it, if not confined to it.

MEASUREMENT OF THE RATE OF RESPIRATION

There are two general methods which may be used in the quantitative estimation of the rate of respiration of vegetable material. One that is employed where facilities are available is to measure in terms of Calories the heat energy released per unit of time and material. The elaborate device for this purpose is known as the respiration calorimeter. The second general method includes the determination of one of the end products of the reaction, carbon dioxid. This method may easily be made decidedly accurate without entailing the assembling of a calorimeter. In using the calorimeter both carbon dioxid respired and heat evolved may, if desired, be determined simultaneously.

Inasmuch as the writers were not provided with a respiration calorimeter suited to this purpose, the carbon dioxid evolved by stored wheat was measured, and from this data the rate of respiration was calculated. Truog's (*1915*) method and absorption tower was used for this purpose, the tower being slightly modified, or rather, added to, in order to adapt it to the present work. The procedure followed and a description of the apparatus has been published by the junior author (*Gurjar, 1917*). To compute the Calories of heat evolved, the factor found by Langworthy and Milner may be employed; 1 gm. of respired carbon dioxid equals 2.6 Calories of heat.

Calcium-chlorid towers were used as respiration chambers. Paraffined wire netting was fitted into the constriction near the base, and on this the grain rested. Rubber connections were made as short as possible, and all stoppers and tubing were thoroughly paraffined to prevent selective absorption of carbon dioxid. In all instances where the same lot of grain was worked with at different moisture contents, the several portions were brought to approximately the desired percentage of moisture by adding water from a burette, at the same time stirring thoroughly. The samples were allowed to stand in sealed jars for three days, in order to insure uniform distribution of the water through the kernels. It had been found by determining the rate of swelling of the kernels that they reached their maximum size in considerably less than three days, and from this it was concluded that the moisture distribution would be complete in the 3-day interval. When the grain was ready to work with, a weighed quantity was sealed into the tower. Samples were taken at this time for the determination of moisture. The amount of grain placed in the tower varied with the moisture content, about 500 gm. being employed in the case of the lower moisture limits, while about 300 gm. were used when the percentage of moisture exceeded 15.5 per cent. In this manner convenient and accurately determinable quantities of carbon dioxid were obtained. After sealing the grain into the glass cylinders, the air was removed and replaced by carbon-dioxid-free air. The towers were then placed in the thermostat, which, except when temperature was the variable, was maintained at 37.8° C. (100° F.).

The period of incubation was fixed at four days, the exact number of hours being noted at the time of removing the respiration chambers from the thermostat. The accumulated carbon dioxid was then removed through the tubulure at the bottom of the tower, carbon-dioxid-free air being simultaneously admitted through the top. The carbon dioxid was absorbed in *N/4* barium hydroxid [$Ba(OH)_2$] solution in the special absorption tower, as described by Gurjar in the above-mentioned paper. The respiration data given in the tables are stated in terms of milligrams of carbon dioxid respired per 24 hours by each 100 gm. of dry matter.

RELATION OF THE MOISTURE CONTENT OF WHEAT TO THE RATE OF RESPIRATION

The observation of Bonnier and Mangin (*1885*) that respiration of living plants varies directly with the humidity of the air might be interpreted to mean that the moisture content of the tissues increased in a humid atmosphere. This increase in turn may have occasioned the rise in the rate of respiration. Maquenne (*1900*) concluded that a reduction in the moisture content of seeds is accompanied by a reduction in the rate of respiration, and Lund (*1894*) discovered that the desiccation of roots and tubers reduced their rate of respiration.

Kolkwitz (*1901*) found that barley grains containing 19 to 20 per cent of moisture respired 3.69 mgm. of carbon dioxid per kilo in 24 hours at summer temperature, while at the same temperature barley containing 14 to 15 per cent of moisture respired 1.4 mgm. per kilo, and 0.35 mgm. per kilo when containing 10 to 12 per cent of moisture.

White (*1909*) found that all cereals gave off appreciable quantities of carbon dioxid when stored in an air-dried condition, the respiration of wheat containing 11.9 per cent of moisture being especially pronounced. Wheat dried for eight days at 45° C. did not respire a determinable quantity of carbon dioxid.

Babcock (*1912*) states that respiration is practically suspended in dry seeds and spores, and is most pronounced when vital processes are most active, as during the germination of seeds. The metabolic water produced as respiration proceeds is believed to play an important part in the vital phenomena of the cells.

Duvel (*1904*), in studying the vitality of stored seeds, observed that the rate of respiration, as indicated by the carbon-dioxid content of the air in the closed container, was increased on increasing the moisture content of the seed. At the same time there was a marked diminution in the percentage of viable seed.

Qvam (*1906*) observed an increased rate of respiration in barley as the percentage of moisture was increased.

Duvel (*1909*) held a lot of corn in storage in an elevator bin. The moisture content averaged 17.8 per cent, and the initial temperature on February 17, 1909, was 36° to 40° F., which increased near the surface of the grain to 133° F. on April 27, 1909. The temperature from the middle to the bottom of the bin was only about 40° F. A portion of the hot corn from the top of the bin was artificially dried to an average of 14.57 per cent of moisture. This was put in a car, and as a control a lot of the cool, undried corn from the same bin was put in another car. The latter had an average moisture content of 17.5 per cent. The dried corn remained for 37 days in as good condition as when put in the car, its temperature rising from 57° to 67° F., or a total increase of 10 degrees. The cool, undried corn began to show signs of deterioration in 23 days, and five days later a point near the surface reached a temperature of 122° F. This indicates the increased tendency of the damp grain to heat in storage.

Shanahan, Leighty, and Boerner (*1910*) examined cargoes of American corn on arrival at European ports and observed an increased tendency to heat and "go out of condition" as the moisture content increased.

Duvel and Duval (*1913*) studied the temperature changes in carloads of corn containing different percentages of moisture. In one experiment

running from March 2 to March 29, 1911, the following temperature changes were observed in cars held on track at Baltimore, Maryland:

Moisture.	Average temperature of corn when—	
	Loaded.	Unloaded.
Per cent.	°*F.*	°*F.*
21.6	40.0	109.7
19.9	40.0	41.5
17.4	40.0	40.5
14.1	40.0	41.3

In a similar experiment, running from May 11, 1911, to June 1 and 3, 1911, corn containing 16.9 per cent of moisture or more was heating when unloaded, while that which contained 13.9 per cent of moisture was still cool. The extent of heating bore a fairly definite relation to the shrinkage or loss in weight of the grain.

Bailey (*1917a*) reported to the Second Interstate Cereal Conference in 1916 the results of storage experiments with wheat at Duluth, Minn. In the cool climate of that city it was found that wheat containing 15.5 per cent of moisture when put in a bin in the fall kept 333 days before it developed a sufficiently high temperature to necessitate turning it, while wheat containing 16.5 per cent of moisture was actively heating in 49 days.

The exact reason for an accelerated respiration with an increased moisture content had not been adequately explained. In the discussion referred to in the paper mentioned in the preceding paragraph an hypothesis was advanced to account for this relation. Moisture in grain may, in the light of recent discoveries in the field of physical chemistry, be assumed to exist as imbibed water in loose combination with the organic colloids. The organic colloids which form the principal constituents of the wheat kernel have the property of imbibing considerable quantities of water and forming elastic gels. The gel swells as the water is increased, although the total volume of the dry colloid plus the added water is diminished. The water-imbibing capacity of the several colloids varies widely, starch having an imbibing capacity materially lower than that of wheat gluten. There is no fixed amount which a given dry colloid will imbibe; thus, gels of varying viscosity can be produced, depending upon the proportion of water present, and other variables, such as temperature, mineral salts, and other substances. The rate of diffusion in a gel varies with the viscosity, as pointed out by Plimmer (*1915, p. 386*). In dilute gels diffusion takes place as in water, while in strong gels the rate is slower. It is probable that in very dry grain the imbibed water is not sufficient to produce a gel in the endosperm structures. The colloidal material there located accordingly does not have a

continuous structure, and the possibilities of diffusion are decidedly reduced under such conditions. The exact percentage of moisture below which this discontinuous structure exists in a normal wheat kernel is not known; it probably varies with the percentage of gluten in the grain since gluten possesses a greater water-imbibing capacity than starch. Increasing the moisture content above the maximum at which discontinuity exists results in the formation of a gel through which diffusion

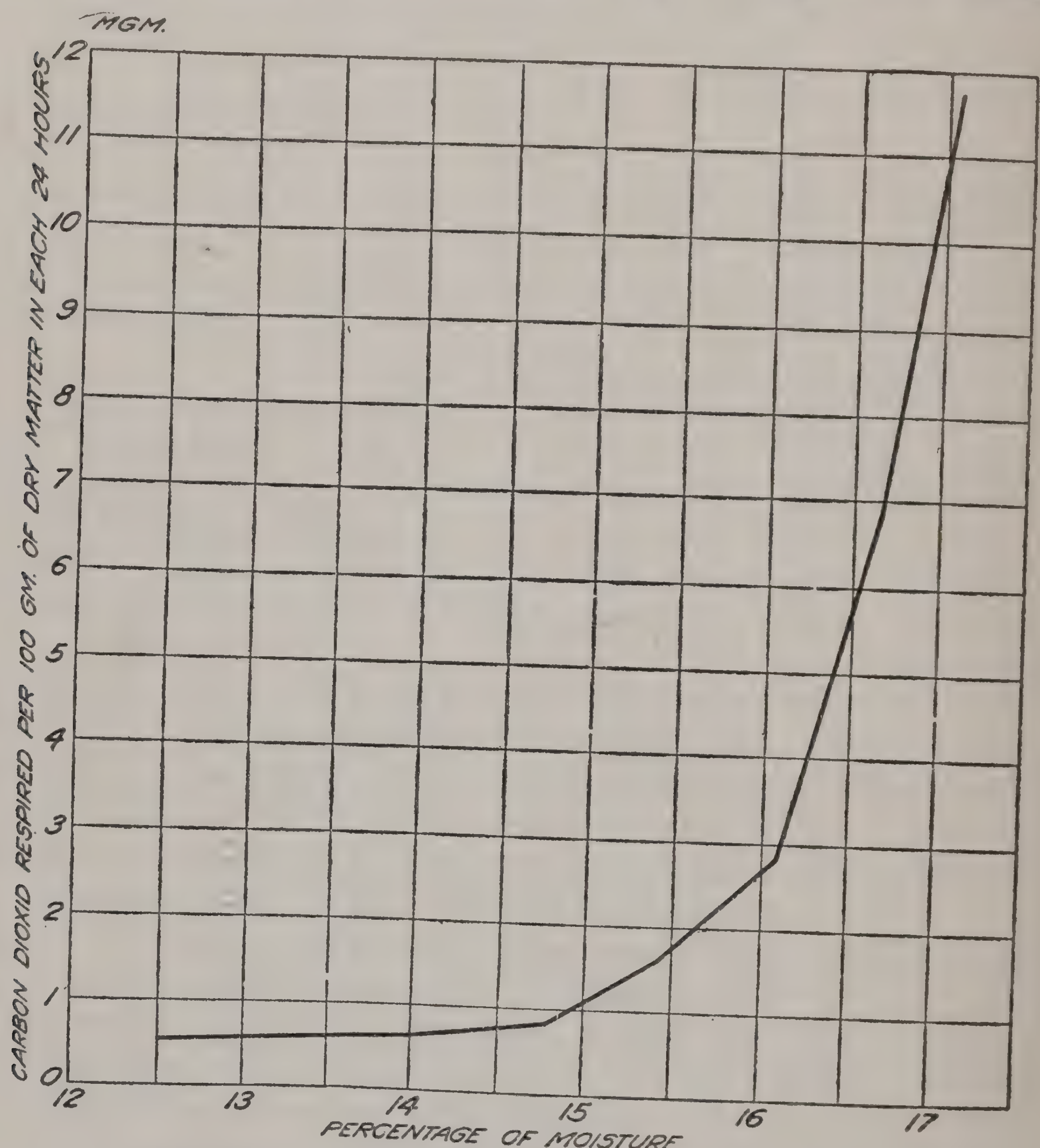

FIG. 1.—Graph showing the relation of the moisture content of wheat to the rate of respiration.

can occur. Further increases in moisture content up to maximum imbibition produce progressively less viscous gels, and correspondingly increase the possible rate of diffusion. Since the rate of respiration in grain doubtless depends in part upon the rate of diffusion between the various kernel structures, it follows that the less viscous the gelatinous material of which the cell contents are composed, the more rapid the production of heat through respiration. To restate, the production of heat is dependent upon the activity of the oxidizing enzyms of the

kernel, the complex phenomenon being known as respiration. The latter is accelerated by an increase in the rate of diffusion, which in turn is dependent upon the existence of a gel, and the relative viscosity of that gel. For these reasons the moisture content of sound grain determines to a considerable extent the rate of respiration and consequent liability of heating when bulk grain is stored.

To determine the relation of moisture content to respiration in stored wheat, a large sample of spring-sown Haynes Bluestem wheat known as Minnesota 169 was obtained. The weight per bushel of the sample was 57½ pounds (26.08 kgm.); the weight per 1,000 average kernels, 24.62 gm.; and it contained 2.21 per cent of nitrogen on the dry basis. It was then divided into several portions, and each portion was brought to a different moisture content, the percentages of moisture ranging from 12.50 to 17.07 per cent. The quantity of carbon dioxid respired per 24 hours by each 100 gm. of dry matter is given in Table I and is shown graphically in figure 1. The rate of increase in respiration is fairly gradual from 12.50 to 14.78 per cent, but after the latter percentage is exceeded the rate is markedly accelerated. The break in the curve occurs when the moisture content slightly exceeds 14.5 per cent, and it is probable that this represents about the maximum percentage of moisture that this class of wheat may safely contain without danger of heating when stored in bulk.

Table I.—*Respiration of Haynes Bluestem (Minnesota 169) wheat,[a] incubated at 37.8° C for four days*

Moisture.	Carbon dioxid respired per 24 hours for each 100 gm. of dry matter.	Moisture.	Carbon dioxid respired per 24 hours for each 100 gm. of dry matter.
Per cent.	*Mgm.*	*Per cent.*	*Mgm.*
12. 50	0. 54	16. 08	2. 88
13. 93	. 65	16. 65	6. 86
14. 78	. 86	17. 07	11. 72
15. 42	1. 62		

[a] Weight per bushel of sample, 57½ pounds. Weight per 1,000 kernels, 24.62 gm. Nitrogen on dry basis, 2.21 per cent.

The acceleration of respiration as the moisture content increases is shown in Table II. These data are based upon the estimated respiration values at even percentages of moisture, and the computed increase in respired carbon dioxid for each increase of 1 per cent of moisture. In computing these data the following formula was employed: $\frac{K_m - K_{m-1}}{K_{m-1}}$ in which K_m represents the respiration value at a particular percentage of moisture, and K_{m-1} represents the respiration value for the same wheat containing 1 per cent of moisture less than K_m. It is evident that the acceleration between 12 and 14 per cent of moisture is very gradual, while it increases markedly after 14 per cent is exceeded.

TABLE II.—*Acceleration of the rate of respiration of hard spring wheat with increasing moisture content*

Formula.	Acceleration at following percentages of moisture.				
	12 to 13	13 to 14	14 to 15	15 to 16	16 to 17
$\frac{K_m - K_{m-1}}{K_{m-1}}$	0.16	0.17	0.66	1.41	3.02

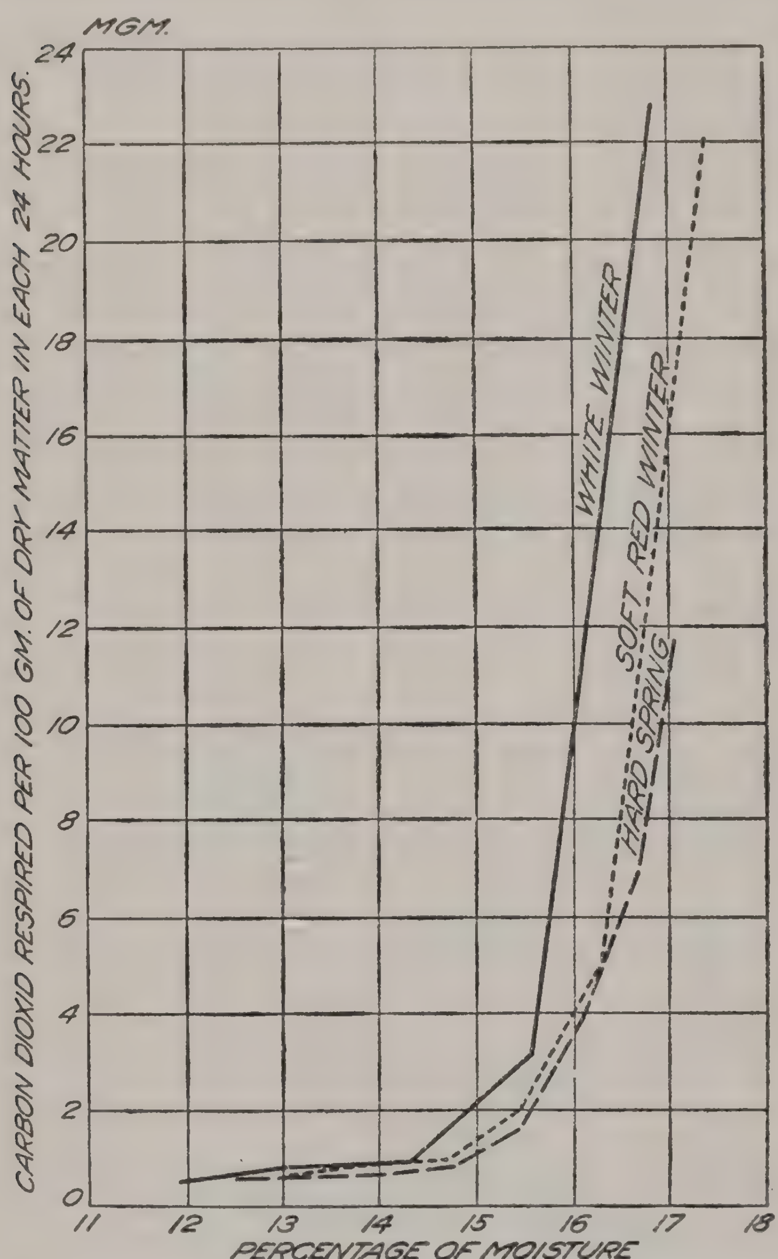

FIG. 2.—Graphs showing the comparative rate of respiration of hard spring, soft red winter, and soft white winter wheat.

RELATION OF THE CONSISTENCY OF THE WHEAT KERNEL TO THE RATE OF RESPIRATION

Reference has already been made to the difference in the relative water-imbibing capacity of the various organic colloids of the wheat kernel. Since starch and gluten constitute a large proportion of the endosperm, their differences in this regard are of principal interest. Simple tests indicate that the water-imbibing capacity of gluten is materially greater than that of starch. In consequence it follows that, as a general rule, those kernels which contain a high percentage of gluten will, at any particular moisture content, be more viscous than will kernels containing a lower percentage of gluten.

The gluten content is also related to the relative consistency or hardness of the wheat berry. In general, the hard, vitreous grains contain a higher percentage of gluten than do the soft, starchy grains. Accordingly we may expect exactly what we find—viz, that the soft wheats are "tougher" and of a lower viscosity at any given moisture content (within the limits found in ordinary commercial grain) than the hard, vitreous wheats.

It is commonly recognized in the grain trade that the keeping qualities of soft wheats are inferior to those of hard wheat. Because of the relation of respiratory activity to rate of diffusion, it should follow that with the same moisture content respiration would proceed more rapidly in a soft than in a hard or vitreous kernel. A sample of soft red winter wheat of the Fultz variety was obtained from the Experiment Station at Columbia, Mo., and another of white winter wheat from Grand Blanc, Mich. The rate of respiration in these soft wheats at different moisture contents was studied, and it was found that, except at the lower percentages of moisture, the rate of respiration was higher in the soft red winter wheat than it was in the hard spring wheat, and still higher in the white winter wheat. These data are given in Tables, III, IV, and V, and graphically in figure 2. As shown by the graph, the curves tend to converge at about 12 per cent of moisture, indicating that at less than this moisture content the discontinuity of endosperm structure referred to above may exist in sound wheats and respiration proceed at the expense of substances in the germ rather than by oxidation of materials which diffuse to it from the endosperm.

It may further be seen that the quantity of heat evolved by hard spring wheat containing 14.5 per cent of moisture, as evidenced by the rate of respiration at that moisture content, was evolved by these soft wheat samples when they contained about 13.6 to 13.8 per cent of moisture. This is of interest, in view of the moisture limits prescribed in the United States Grain Standards for wheat, which are 14½ per cent for No. 2 hard spring, and 13 per cent for No. 2 soft red winter, and the same for common white and white club wheat.

TABLE III.—*Respiration of soft red winter wheat* [a] *from Missouri, incubated at 37.8°C. for four days*

Moisture.	Carbon dioxid respired per 24 hours for each 100 gm. of dry matter.	Moisture.	Carbon dioxid respired per 24 hours for each 100 gm. of dry matter.
Per cent.	*Mgm.*	*Per cent.*	*Mgm.*
13.07	0.65	15.45	2.00
13.63	.80	16.37	5.06
14.70	.95	17.40	22.03

[a] Weight per bushel of sample, 61 pounds. Weight per 1,000 kernels, 29.97 gm. Nitrogen on dry basis, 1.54 per cent.

TABLE IV.—*Respiration of white winter wheat*[a] *from Michigan, incubated at 37.8°C. for four days*

Moisture.	Carbon dioxid respired per 24 hours for each 100 gm. of dry matter.	Moisture.	Carbon dioxid respired per 24 hours for each 100 gm. of dry matter.
Per cent.	*Mgm.*	*Per cent.*	*Mgm.*
11.94	0.48	15.57	3.20
13.04	.60	16.83	22.77
14.32	.89		

[a] Weight per bushel of sample, 59 pounds. Weight per 1,000 kernels, 37.73 gm. Nitrogen on dry basis, 1.53 per cent.

TABLE V.—*Interpolated quantity of carbon dioxid respired per unit of time and material, at even percentages of moisture*

Class of wheat.	Carbon dioxid respired per 24 hours for each 100 gm. of dry matter.					
	12 per cent moisture.	13 per cent moisture.	14 per cent moisture.	15 per cent moisture.	16 per cent moisture.	17 per cent moisture.
	Mgm.	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*
Hard spring	0.50	0.58	0.68	1.13	2.72	10.73
Soft red winter		.63	.81	1.37	3.84	15.51
White winter	.49	.60	.83	4.15	9.85	25.18

RELATION OF THE RELATIVE PLUMPNESS OF THE WHEAT KERNEL TO THE RATE OF RESPIRATION

It is generally recognized that the velocity of enzym action conforms quite closely to the law of mass action. Thus any condition which affects the quantity of either the substrate or the enzym will cause variations in the rate of the reaction. Since respiration is occasioned by enzyms, the rate of respiration of the wheat kernel should vary with these conditions.

A shriveled condition of the wheat kernel is due generally to factors operating during the later stages of kernel development. The translocation of reserves to the kernel is interfered with by rust, drouth, desiccation by hot winds, or some other agency; and an incomplete filling of the endosperm results. According to Brenchley (*1909*), the germ portion of the kernel is developed earlier than the endosperm, and tends to escape injury from the agency causing shrunkenness of the endosperm more than does the latter. The diminished size and weight of the shriveled kernel is therefore due principally to the decreased quantity of endosperm.

The enzymic activities of the kernel seem to be mainly invested in the embryo. This was discussed in one of the foregoing paragraphs. In the embryo of the shriveled wheat berry the enzyms are probably represented practically as they are in the normal kernel. In instances where the average weight of the individual kernel is only about half the normal,

it follows that there is approximately twice the enzymic activity per unit of mass than that shown by normal wheat. The normal spring wheat used by the writers weighed 24.62 gm. per 1,000 average kernels. A shriveled sample of the same type of wheat was obtained which weighed 11.73 gm. per 1,000 kernels, or less than half the weight of the normal. The rate of respiration of these two lots was compared, and, as shown in figure 3, the respiratory activity of the shriveled sample decidedly exceeded that of the normal, or plump wheat. Thus the quantity of carbon dioxid respired by the latter when it contained 14.5 per cent of moisture was respired by the shriveled wheat used when it contained only 12.8 per cent of moisture. The curves tend to converge at moisture contents slightly below 12 per cent.

The respiration data for the shriveled sample are given in Table VI, while in Table VII is shown the interpolated quantity of carbon dioxid respired by the normal and shriveled wheats at even percentages of moisture.

TABLE VI.—*Respiration of shriveled spring wheat,[a] incubated at 37.8° C. for four days*

Moisture.	Carbon dioxid respired per 24 hours for each 100 gm. of dry matter.	Moisture.	Carbon dioxid respired per 24 hours for each 100 gm. of dry matter.
Per cent.	*Mgm.*	*Per cent.*	*Mgm.*
12. 68	0. 75	15. 68	4. 50
13. 19	. 94	16. 09	10. 51
14. 29	1. 38	16. 44	16. 92
15. 30	3. 02	16. 80	21. 65

[a] Weight per bushel of sample, 47½ pounds. Weight per 1,000 kernels, 11.73 gm. Nitrogen on dry basis, 2.03 per cent.

TABLE VII.—*Interpolated quantity of carbon dioxid respired per unit of time and material at even percentages of moisture*

Class of wheat.	Carbon dioxid respired per 24 hours for each 100 gm. of dry matter.					
	12 per cent moisture.	13 per cent moisture.	14 per cent moisture.	15 per cent moisture.	16 per cent moisture.	17 per cent moisture.
	Mgm.	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*
Plump spring wheat..........	0. 50	0. 58	0. 68	1. 13	2. 72	10. 93
Shriveled spring wheat........	. 65	. 88	1. 26	2. 54	9. 41	22. 65

SOUNDNESS OF THE WHEAT KERNEL IN ITS RELATION TO THE RATE OF RESPIRATION.

A form of unsoundness recognized as such by the grain trade and frequently occurring in spring wheat is the frosted condition. This results from the freezing of the plants before the grain is matured and desiccated. The plants usually thaw later, and, while the protoplasm

is killed or disorganized and its synthetic activities reduced or destroyed, certain hydrolytic enzyms are activated, and hydrolysis or splitting of certain of the kernel constituents ensues. As a result, there is an accumulation of the split products of starch and proteins, particularly dextrose and amino acids. It is probable that the extent of starch hydrolysis by amylases depends in large part upon the percentage of moisture in the grain after it thaws out. If the kernels are nearly dry, less change will occur than if the kernels contain considerable moisture.

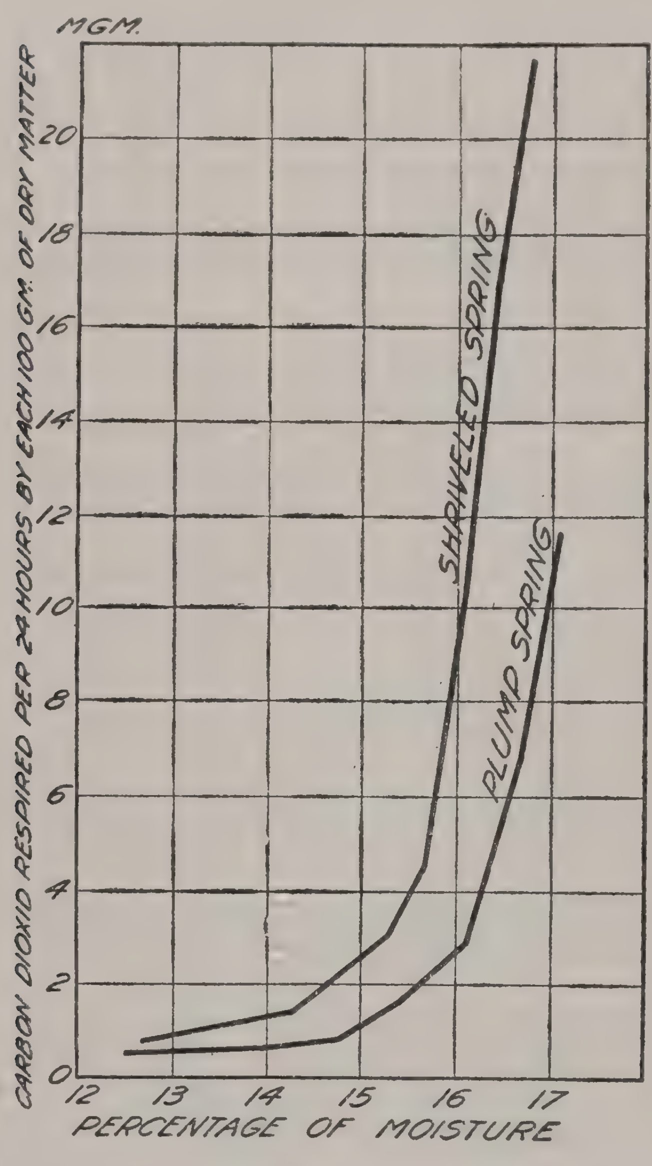

FIG. 3.—Graphs showing the rate of respiration of shriveled wheat and of plump wheat of the same class.

The dextrose which thus accumulates in the kernel is presumably available as substrate for the respiratory enzyms. In accordance with the law of mass action, a greater concentration of substrate should accelerate the rate of respiration. There is an additional factor in the case of frosted wheat that would also tend to result in an increased rate of respiration at any particular percentage of moisture. The hydrolysis of the gluten subsequent to thawing results in products having a materially lower water-imbibing capacity than the normal gluten. In fact, the amino acids formed are not colloids, and form true solutions. Consequently the relative viscosity of frosted grain at any moisture content will be less than in normal grain, the difference depending upon the extent of hydrolysis.

To ascertain the effect of frosting upon the rate of respiration, two samples of commercial wheat containing frosted kernels were secured. These were marked "moderately frosted" and "badly frosted," respectively. The respiratory activity of these frosted samples was determined with five different percentages of moisture present. Tables VIII and IX show the respiration data of these two lots, while in Table X are given the interpolated values at even percentages of moisture in comparison with sound spring wheat. The same data are shown graphically in figure 4. There is a slight overlapping of the curves for the

moderately and badly frosted samples between 14.5 and 15.5 per cent of moisture, the reason for which is not clear. The discrepancy is not great, and the similarity of the curves indicates that possibly our judgment was at fault in describing the two lots of frosted wheat as "moderately" and "badly" damaged. The decided differences in the rate of

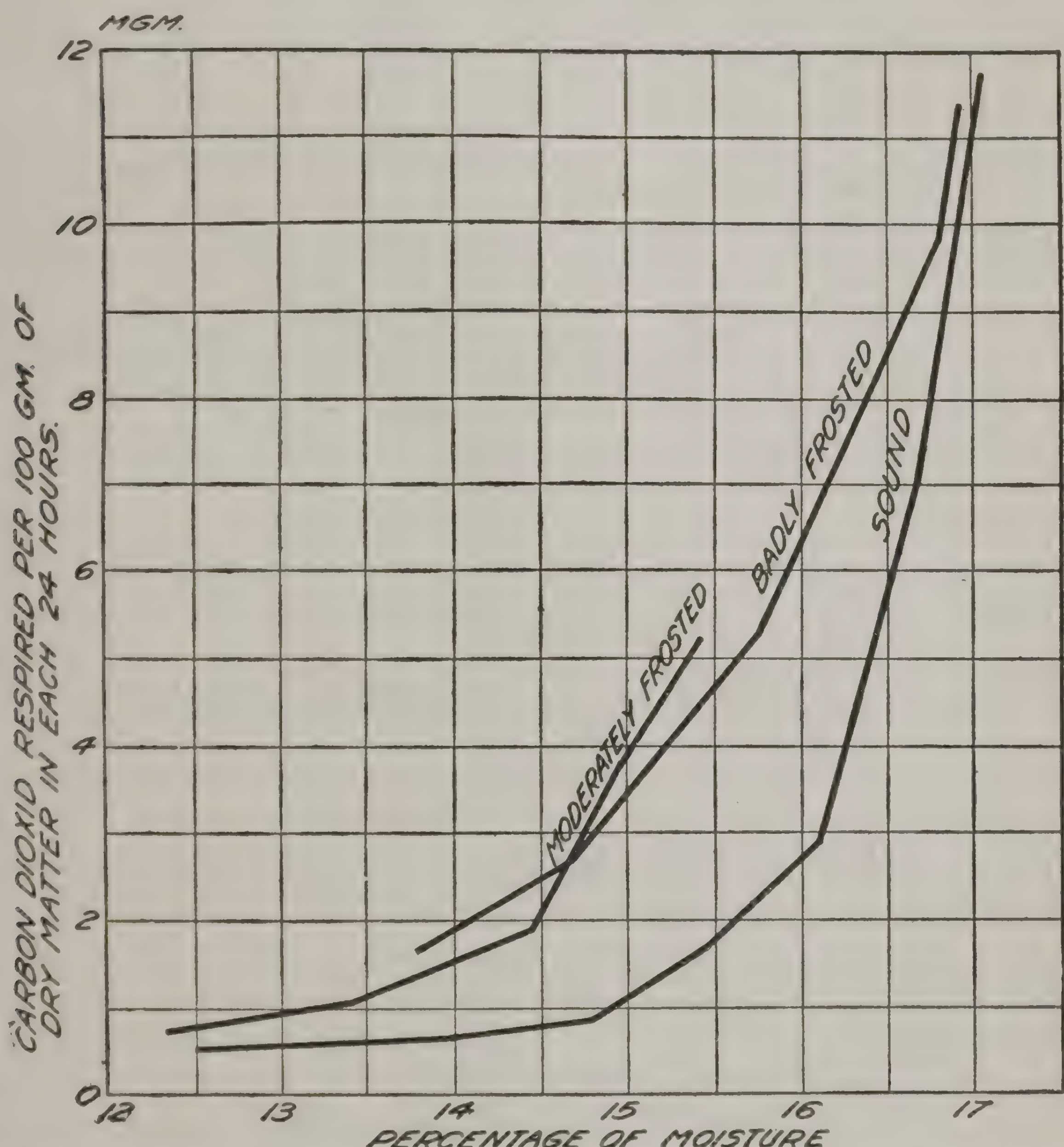

FIG. 4.—Graphs showing the rate of respiration of frosted wheat and sound wheat of the same class.

respiration of frosted and of normal wheat containing up to 16.5 per cent of moisture substantiates what had previously been empirically observed by the senior author (*Bailey, 1917b*)—viz, that frosted wheat tends to heat more readily when stored than does sound wheat containing the same percentage of moisture.

TABLE VIII.—*Respiration of moderately frosted wheat,[a] incubated at 37.8° C. for four days*

Moisture.	Carbon dioxid respired per 24 hours for each 100 gm. of dry matter.	Moisture.	Carbon dioxid respired per 24 hours for each 100 gm. of dry matter.
Per cent.	*Mgm.*	*Per cent.*	*Mgm.*
12.32	0.74	14.95	3.75
13.38	1.04	15.42	5.21
14.44	1.89		

[a] Weight per bushel of sample, 58 pounds. Weight per 1,000 kernels, 36.94 gm. Nitrogen on dry basis, 2.02 per cent.

TABLE IX.—*Respiration of badly frosted wheat,[a] incubated at 37.8° C. for four days*

Moisture.	Carbon dioxid respired per 24 hours for each 100 gm. of dry matter.	Moisture.	Carbon dioxid respired per 24 hours for each 100 gm. of dry matter.
Per cent.	*Mgm.*	*Per cent.*	*Mgm.*
13.79	1.63	16.81	9.82
14.67	2.64	16.95	11.40
15.74	5.24		

[a] Weight per bushel of sample, 53 pounds. Weight per 1,000 kernels, 22.52 gm. Nitrogen on dry basis, 2.10 per cent.

TABLE X.—*Interpolated quantity of carbon dioxid respired per unit of time and material, at even percentages of moisture*

Class of wheat.	Carbon dioxid respired per 24 hours for each 100 gm. of dry matter.					
	12 per cent moisture.	13 per cent moisture.	14 per cent moisture.	15 per cent moisture.	16 per cent moisture.	17 per cent moisture.
	Mgm.	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*
Sound spring wheat	0.50	0.58	0.68	1.13	2.72	10.93
Moderately frosted	.65	.94	1.52	3.90	....	....
Badly frosted	1.12	1.20	1.87	3.46	6.35	11.97

TABLE XI.—*Respiration of several samples of frosted wheat from car lots, and containing varying percentages of moisture. Incubated at 37.8° C. for four days*

Laboratory No.	Moisture.	Weight per bushel.	Weight per 1,000 kernels.	Nitrogen on dry basis.	Carbon dioxid respired per 24 hours for each 100 gm. of dry matter.	Interpolated respiration of sound wheat of same water content.
	Per cent.	*Pounds.*	*Gm.*	*Per cent.*	*Mgm.*	*Mgm.*
G122	14.30	60½	28.36	2.23	1.71	1.30
G123	14.82	61	28.64	2.20	2.34	1.73
G118	16.16	56½	23.56	2.27	8.71	6.86
G102	16.19	57	28.88	2.00	8.24	7.15

Several wheat samples taken from car lots by samplers of the State Grain Inspection Department were frosted, and the respiratory activity

of four of these was measured. In every case the rate of respiration was greater than in sound wheat of the same moisture content which was taken from car lots at the same time. The tests of these frosted samples are shown in Table XI, the last column of which shows the interpolated respiration data of sound wheat.

THE PERIOD OF DAMPNESS AS INFLUENCING THE RATE OF RESPIRATION OF WHEAT

When damp wheats are stored, the excess moisture which is responsible for their dampness has been present for varying lengths of time. This is called for the purposes of this discussion the "period of dampness."

In connection with these studies a series of wheat samples was taken from car lots by the State Grain Inspection Department between the 15th and 27th of March, which contained varying percentages of moisture. The respiratory activity of each of these was determined, and on plotting a curve it was observed that these samples gave materially higher values than the samples which had been dampened and allowed to stand for three days before measuring the rate of respiration. The curves tend toward convergence at a moisture content of 12 per cent. These data are given in Tables XII and XIII and are shown graphically in figure 5.

TABLE XII.—*Respiration of natural hard spring wheat collected from car lots, and incubated at 37.8° C. for four days*

Laboratory No.	Moisture.	Weight per bushel.	Weight per 1,000 kernels.	Nitrogen in dry matter.	Carbon dioxid respired per 24 hours for each 100 gm. of dry matter.
	Per cent.	*Pounds.*	*Gm.*	*Per cent.*	*Mgm.*
G128	12.47	62	30.56	2.14	0.61
G127	13.11	63	32.94	2.16	.75
G124	14.70	58½	24.60	2.05	1.49
G120	15.51	52	26.92	2.31	3.26
G104	15.73	59	28.64	2.47	3.94
G103	16.00	59	25.72	2.37	5.69
G107	16.53	56	22.88	2.25	10.65
G109	16.90	51	....	....	13.86

TABLE XIII.—*Interpolated quantity of carbon dioxid respired per unit of time and material, at even percentages of moisture*

Class of wheat.	Carbon dioxid respired per 24 hours for each 100 gm. of dry matter.					
	12 per cent moisture.	13 per cent moisture.	14 per cent moisture.	15 per cent moisture.	16 per cent moisture.	17 per cent moisture.
	Mgm.	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*
Freshly dampened wheat	0.50	0.58	0.68	1.13	2.72	10.73
Natural wheat	.51	.73	1.15	2.14	5.69	15.03

Since the principal difference between the samples dampened by the writers and the damp grain obtained from the freight cars was the length of the period of dampness, an experiment was conducted to ascertain the extent to which that variable affected the rate of respiration. Two samples of Bluestem wheat that had been dampened some time previous

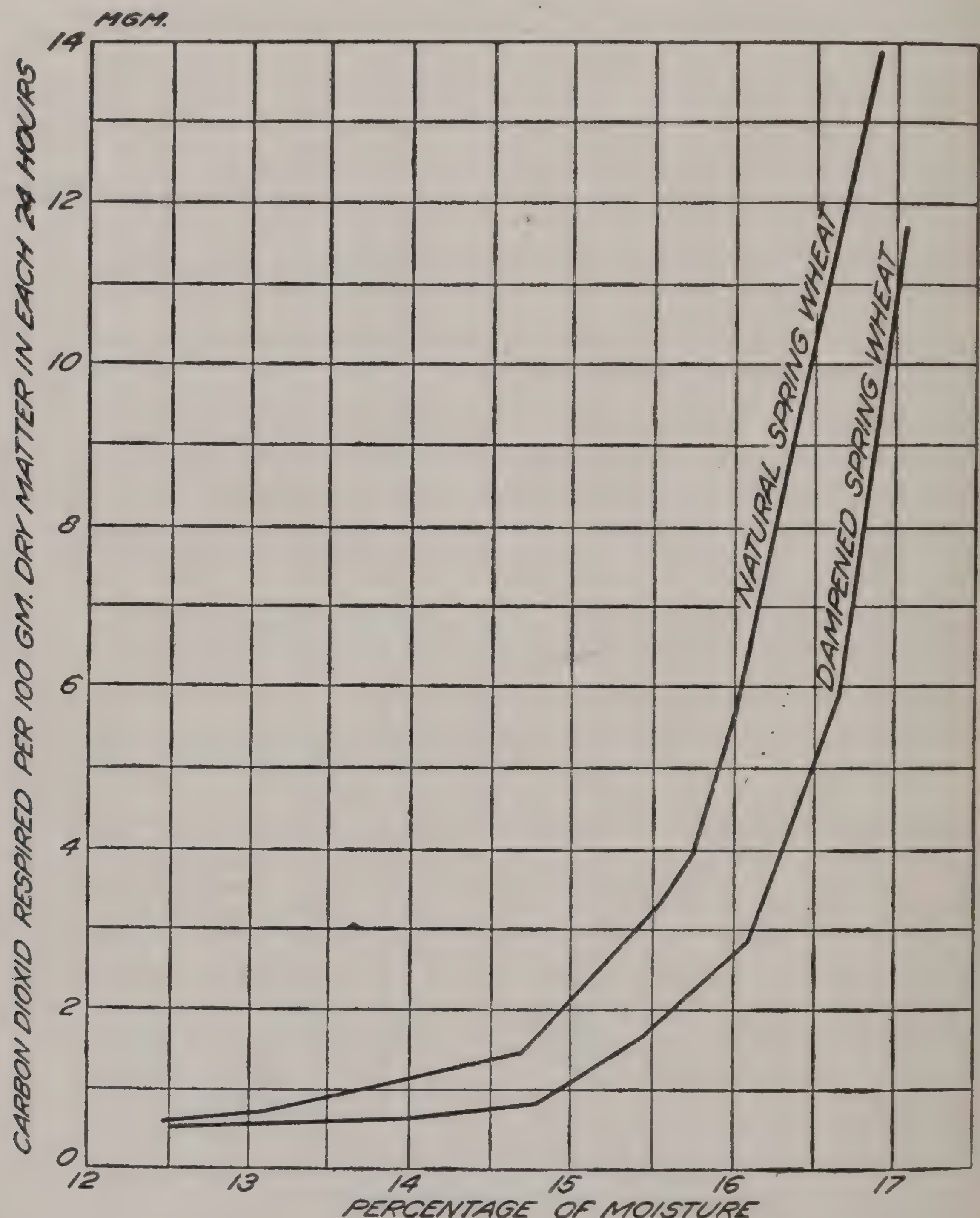

FIG. 5.—Graphs showing the comparative respiratory activity of naturally damp wheats and of wheats dampened in the laboratory three days before they were incubated.

and stored at a temperature of about 25° C. in the laboratory vault were incubated in the usual manner at 37.8° C. with the results shown in Table XIV. The quantity of carbon dioxid respired by the sample containing 15.21 per cent of moisture, which was stored for 55 days after it was dampened, was about four times as great as that respired by freshly dampened wheat of the same moisture content, while that from

the sample containing 15.71 per cent of moisture, which was stored for 108 days, was about eight times as great as for freshly dampened wheat containing that percentage of moisture.

TABLE XIV.—*Respiration of dampened wheat after storage at about 25° C. Incubated at 37.8° C. for four days*

	Lot A.	Lot B.
Moisture........per cent..	15.21	15.72
Number of days stored........	55	108
Carbon dioxid respired per 100 gm. of dry matter in each 24 hours........mgm..	5.31	17.00
Carbon dioxid respired per 100 gm. of dry matter in each 24 hours, of same lot of wheat 4 days after dampening........mgm..	1.35	2.17
Carbon dioxid respired by naturally damp wheat from car lots, of same moisture content........mgm..	2.59	3.85

Table XIV also shows a decided difference in the comparative rate of respiration of wheat stored for a time in a warm room and that obtained from freight cars. The latter had no doubt been damp for a longer time than that which was dampened and held in the room. These data suggest that not only does the period of dampness affect respiration, but the conditions of storage may have an equally important effect. The grain taken from cars was cold at the time, and had probably been exposed to the cold atmospheric conditions of the preceding winter months. If, as postulated by the writers, dextrose tends to accumulate in the stored damp grain the rate of accumulation would depend upon the temperature of the grain as well as upon the time elapsed. Accordingly there may have been more substrate (dextrose) for the respiratory enzyms in the grain which had been stored in the warm room than there was in that stored out of doors during the winter months. A series of experiments with both the period of dampness and the temperature of storage as variables have accordingly been begun, and the results will be reported later in another publication.

INFLUENCE OF TEMPERATURE ON THE RESPIRATION OF STORED WHEAT

Pfeffer (*1878*) observed that the intensity of respiration increases with the temperature until the latter begins to injure all the vital processes. Hoff (*1896*, p. *125*) stated that the rate of respiration increases two or three times for each 10-degree rise in temperature in accordance with the usual rule of chemical reactions. Ziegenbein (*1893*) found that temperatures above 45° C. were injurious

Clausen (*1890*) studied the respiration of germinating wheat at different temperatures and found the optimum to be about 40° C. The rate of respiration was 2.86 times as great at 10° as at 0° and 1.09 times as great

at 40° as at 30°. The average increase between 0° and 40° was 2.71 times for each 10-degree increase in temperature.

Qvam (*1906*) found the rate of respiration increased up to at least 45° C., which was apparently the highest temperature at which observations were made. The grain was very moist, 100 gm. of water having been added to 200 gm. of grain.

Matthaei (*1905*) investigated the respiration of cherry-laurel leaves at different temperatures and reported an increasing rate of respiration between 5.8° and 33.1° C. At 5.8° 2 gm. of green leaves respired 0.1 mgm. of carbon dioxid per hour, and at 33.1° the rate was 1.35 mgm. per hour.

The experiments of Duvel and Duval (*1913*) with shelled corn indicate the relation of air temperatures to the heating of such material. Shelled corn in transit and on track containing 16.9 per cent of moisture began to heat and go out of condition between May 11 and June 3, 1911, while in the period from December 24, 1910, to January 20, 1911, corn containing 22 per cent of moisture gained only a few degrees in temperature. The purely physical factor of heat loss into the cold winter atmosphere, of course, served to reduce the rate of rise in temperature, but there was probably a diminished rate of evolution of heat as well.

Attention has been called by Bailey (*1917a*), to the influence of atmospheric temperatures upon the rate of heating of damp wheat. A lot of wheat containing 16.5 per cent of moisture required but 11 days to increase in temperature from 70° to 80° F., when the mean air temperature was 62.1° F., while later in the year, when the mean air temperature was 44.3° F., another lot of wheat containing the same percentage of moisture was stored 49 days before its temperature increased to the same extent.

Another comparison of the influence of temperature on the rate of heating in storage was afforded by two lots of wheat put into bins at about the same time and containing nearly the same percentage of moisture. The initial temperature of one lot was 74° F., and of the other 70°. The latter required over five times as long to reach a temperature of 80° as did the former, owing to the slow increase in temperature at the outset as contrasted with the rapid rate of increase as the temperature mounted higher.

To ascertain the relation of temperature to the rate of respiration in stored grain, a large sample of Minnesota 169 Bluestem wheat was dampened until it contained 14.96 per cent of moisture. Aliquots of this sample were sealed in glass jars and kept in a refrigerator until they were used. This was done to minimize enzymic changes in the grain. The necessary quantities were drawn from the refrigerator for incubation at the several temperatures.

The lowest temperature at which observations were made was 4° C., and since the increase in rate of respiration between 4° and 25° was rela-

tively small, no intermediate temperatures were employed. Regular increases in the temperature of the thermostat by 10-degree intervals were then made until the respired carbon dioxid showed a marked diminution.

The data in Table XV and the graph in figure 6 show that the rate of respiration increased to a maximum at 55° C. This is therefore the temperature at which the most rapid evolution of heat would occur. A discoloration of the seed coat of the wheat kernel begins to show on some kernels at about 35° C., while at 55° the whole mass is of a mahogany color. At 65° the respiratory enzyms have been partially but not

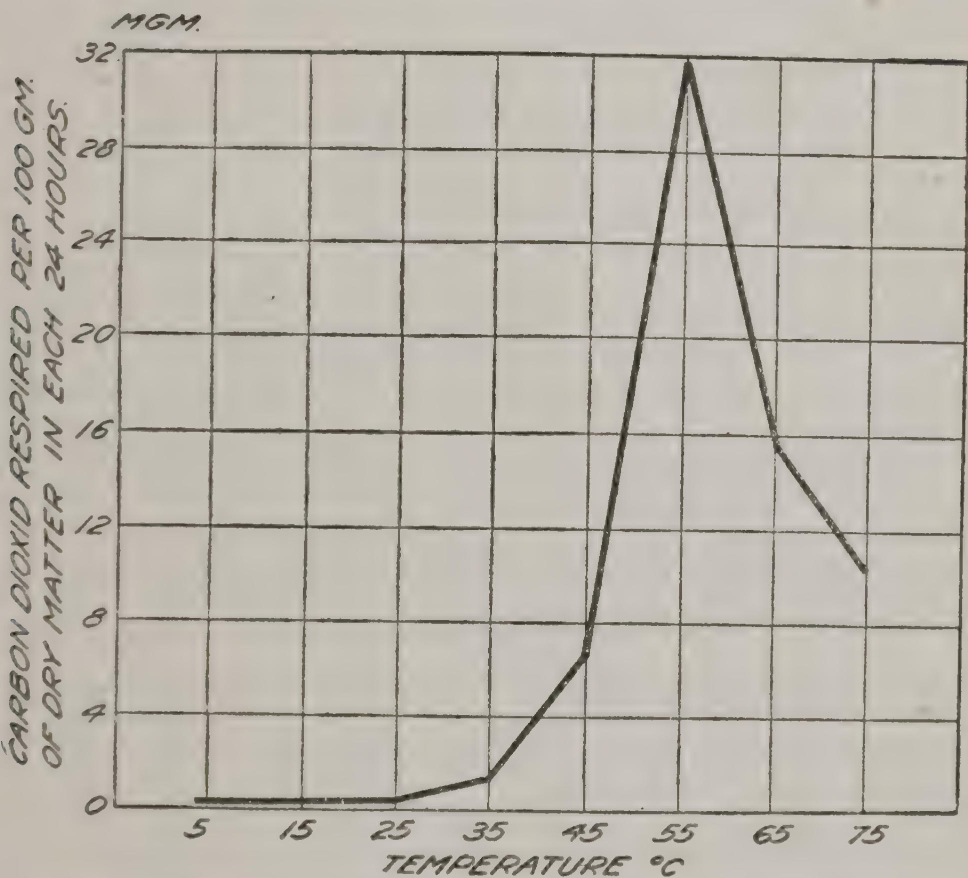

FIG. 6.—Graph showing the relation of temperature to the rate of respiration.

wholly inactivated, while at 75° this inactivation has proceeded still further, and some roasting of the grain has occurred.

TABLE XV.—*Respiration of hard spring wheat at different temperatures*

Temperature.	Carbon dioxid respired per 24 hours for each 100 gm. of dry matter.	Temperature.	Carbon dioxid respired per 24 hours for each 100 gm. of dry matter.
°C.	*Mgm.*	°C.	*Mgm.*
4	0.24	55	31.73
25	.45	65	15.71
35	1.30	75	[a] 10.28
45	6.61		

[a] A part of this carbon dioxid may have resulted from roasting the grain.

The proportional change in respiration for each 10-degree rise in temperature is shown in Table XVI. The data in this table were obtained by employing the conventional formula $\frac{V_{t+10}}{V_t}$ in which V_t represents the rate of respiration at the specified temperature, and V_{t+10} represents the rate at a temperature 10 degrees higher. The values at 5° and 15° were computed by integrating the actual data obtained at 4° to 25° and 35° C.

TABLE XVI.—*Acceleration of respiration for each 10-degree increase in temperature*

t.	$\frac{V_{t+10}}{V_t}$	t.	$\frac{V_{t+10}}{V_t}$
° C.		° C.	
5	1.16	35	5.08
15	1.55	45	4.80
25	2.89	55	.49

INFLUENCE OF ACCUMULATED CARBON DIOXID UPON RESPIRATION

Müntz (*1881*) observed that ten times as much carbon dioxid was respired when grain had access to free air as when sealed air-tight and that the yield of carbon dioxid was much greater after the moisture exceeded 13 to 14 per cent.

Mangin (*1896*) found the evolution of carbon dioxid and absorption of O_2 to be reduced when germinating seeds were put in air containing up to 5 per cent of carbon dioxid. The $\frac{CO_2}{O_2}$ ratio was increased, indicating that absorption of oxygen was diminished more than the evolution of carbon dioxid.

Duvel (*1904*), Babcock (*1912*), Barnes and Grove (*1916*), and others have called attention to the reduced vitality and germination of seeds stored in carbon dioxid, or in tight containers in which the respired carbon dioxid accumulated.

In the case of grain stored under ordinary commercial conditions it follows that the oxygen in the space surrounding the kernels must be replaced with respired carbon dioxid. The rate of such replacement will, of course, hinge upon the factors influencing the rate of respiration. To determine the relative change in the respiration of grain stored in a tight container in which the respired carbon dioxid must accumulate, the following experiment was conducted: A sample of wheat containing 15.05 per cent of moisture was divided into four portions, which were incubated in the usual manner at 37.8° C. At the end of 1, 4, 8, and 12 days a cylinder was removed and the respired carbon dioxid determined. The rate of respiration for the first day and the average rate for each of the 4-day periods are shown in Table XVII and graph-

ically in figure 7. Both table and graph show plainly that the rate of respiration is reduced by the accumulated carbon dioxid, and it is probable that a further reduction in respiratory activity would result if the proportion of carbon dioxid were increased. This fact has an important bearing on commercial practices. It indicates that stored grain should not be disturbed so long as its temperature does not exceed that of the atmosphere, since exposing it would serve to ventilate the grain and thus

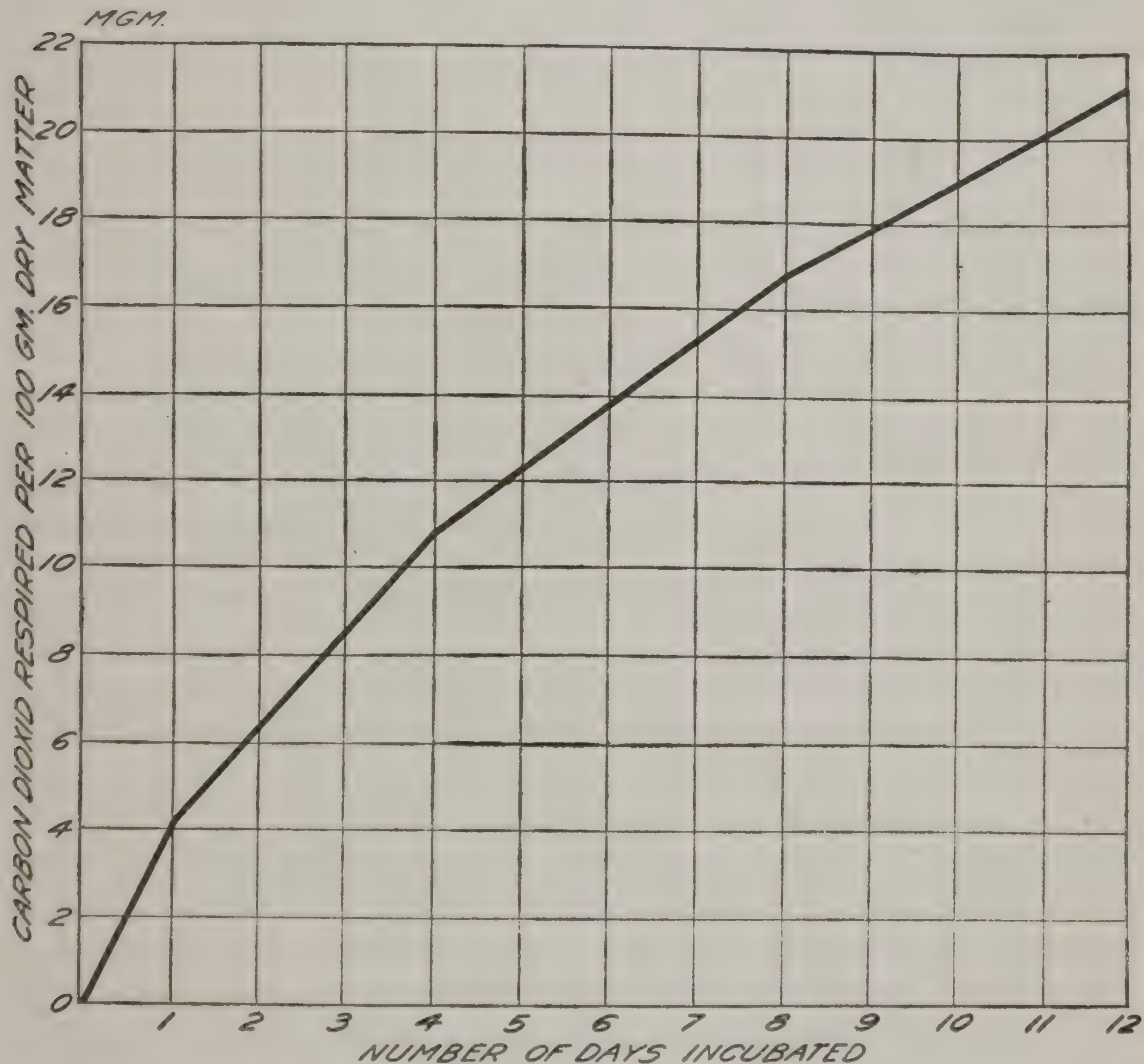

Fig. 7.—Graph showing the rate of respiration during successive intervals when the respired carbon dioxid was permitted to accumulate in the mass of grain.

remove the carbon dioxid. On returning such grain to the bin after aerating it the rate of respiration would be increased over that of the grain which was not handled and exposed, since the rate of respiration of the unaerated grain has been depressed, owing to the accumulated carbon dioxid. The aerated grain will accordingly heat more rapidly than before aerating, unless its temperature has been materially reduced by exposing it to the air.

TABLE XVII.—*Rate of respiration per day for several successive periods*

Period.	Carbon dioxid respired per 100 gm. of dry matter.
	Mgm.
First day	4. 11
Average rate per day for first 4-day period	2. 68
Average rate per day for second 4-day period	1. 49
Average rate per day for third 4-day period	1. 11

RESPIRATION IN OXYGEN-FREE ATMOSPHERE

That respiration may occur in the absence of oxygen was first discovered by Rollo in 1798 (*Hill, 1913*) in working with barley grains. Other investigators confirmed this observation, and Pfeffer (*1878*) suggested the term "intramolecular respiration" for this class of phenomena. Takahashi (*1905*) reported that rice can germinate in water without the presence of sugar and in the entire absence of any air. Hill (*1913*) determined the rate of respiration of water-soaked and sterilized wheat in air, nitrogen, and hydrogen. The decrease in respiration in a continuous current of hydrogen and nitrogen below that in a continuous current of air was about 50 per cent in seeds sterilized in alcohol and about 80 per cent in seeds sterilized in formalin.

It appeared desirable to determine the effect of the elimination of oxygen upon the respiration of stored wheat, and to this end the following experiment was conducted: Two lots of wheat containing 15.6 per cent and 17.6 per cent of moisture respectively were secured. A portion of each lot was sealed in cylinders, the air was removed and replaced by nitrogen. These lots and controls in ordinary atmospheric air freed from carbon dioxid were then incubated for four days at a temperature of 23.9° C. The data in Table XVIII show the rate of respiration in the oxygen-free atmosphere to have been reduced to about two-fifths of that in a normal atmosphere.

TABLE XVIII.—*Comparative rate of respiration in oxygen-free and normal atmosphere*

Moisture.	Carbon dioxid respired per 100 gm. of dry matter in each 24 hours.	
	Oxygen-free atmosphere.	Normal atmosphere.
Per cent.	*Mgm.*	*Mgm.*
15. 6	0. 43	1. 10
17. 6	2. 80	6. 80

CONCLUSIONS

(1) Deductions from these investigations support the findings of earlier investigators that spontaneous heating in damp grain is occasioned by the biological oxidation of dextrose and similar sugars, chiefly in the germ or embryo of the kernel.

(2) Moisture is one of the determining factors in respiration. It establishes the comparative rate of diffusion between the several kernel structures. Any gain in the moisture content of the kernel accordingly increases the rate of diffusion and, simultaneously, the rate of respiration. The increase is gradual and fairly uniform until the moisture exceeds 14.5 per cent, in the case of plump spring wheat, when it is markedly accelerated.

(3) Density of the wheat kernel generally parallels the gluten content. Gluten possesses the property of imbibing more water than starch, and thus varying percentages of gluten result in varying degrees of viscosity at the same moisture content. The relative viscosity affects the rate of diffusion and this in turn directly affects the rate of respiration. The soft, starchy wheats thus respire more rapidly than hard, vitreous wheats containing the same percentage of moisture.

(4) Plumpness of the wheat kernel affects the rate of respiration, as shown by contrasting plump and shriveled grain. The shriveled wheat respired two to three times as much as did the plump wheat at moisture contents above 14 per cent. At percentages of moisture below 14 per cent the difference is not very marked. The high acceleration of respiration in shriveled wheat containing more than 14 per cent of moisture is attributed to the higher ratio of germ to endosperm and hence the larger percentage of enzym to substrate as compared with plump wheat.

(5) The period of dampness—that is, the length of time the excess moisture has been present in the wheat—bears a relation to the rate of respiration. This is shown by comparing the respiration of freshly dampened wheat with that of naturally damp grain and with grain that had been dampened and stored for varying lengths of time. The curve of respiration diverges from that of freshly dampened wheat when the moisture content exceeds 12 per cent, and this divergence is more marked after 13 per cent of moisture is reached. In the case of wheat dampened and stored, the quantity of carbon dioxid respired varies directly with the number of days the wheat remained in storage. The temperature at which the grain is stored affects the rate of diastatic action, thus increasing the quantity of substrate available to the respiratory enzyms. This is indicated by the greater rate of respiration of wheat stored at room temperature than that stored at the outdoor temperature during the winter months.

(6) Unsoundness of wheat caused by the freezing of the unripe plant results in higher respiratory activity in the threshed grain. This was

shown by comparing moderately and badly frosted wheats with sound wheat. The frosted wheat respired more vigorously than the sound wheat. This was attributed to the arresting of the synthetic processes on freezing, and subsequent activities of the hydrolytic enzyms on thawing of the frozen wheat. The accumulation of glucose as the result of starch hydrolysis furnishes larger quantities of substrate to the respiratory enzyms.

(7) Increasing temperatures accelerate the rate of respiration until 55° C. is reached. As the temperature rises the diastatic action upon starch increases. A point is reached, however, at which the enzym activity diminishes.

(8) Accumulation of carbon dioxid in the respiration chamber decreases the rate of respiration. The mean rate by four-day intervals is highest for the first four days and diminishes materially in successive periods.

(9) Respiration is reduced in an oxygen-free atmosphere, the ratio to that occurring in a normal atmosphere being about 1 to 2.5.

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EFFECTS OF MISTLETOE ON YOUNG CONIFERS

By James R. Weir

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During the years 1911 to 1917 the writer has been engaged in the investigation of the injurious effects of mistletoes of the genus Razoumofskya (Arceuthobium) on conifers. A part of these investigations have already been published.[1] During the course of the work, a number of studies were made which have not yet been reported. It seems that these studies are of sufficient importance to be presented at this time.

The fact that these parasites are a cause of suppression [2] in forest trees is readily appreciated after the injury has become acute. In middle-aged and older trees where accumulated injury has resulted in small diameters and broomed scraggly crowns with reduced leaf surface, the effect of the parasites is quite evident (Pl. 37, A). The effects of mistletoe on its host is by no means so apparent on trees ranging in age from 4 to 10 years. Trees which have become infected early in life may begin to react in marked degree, but the extent of the suppression is not always apparent to the eye. It may be noticed that the branches of young trees are broomed along with other types of infection of a more general nature, but the retardation of the excurrent or elongated growth of the main stem may not be readily recognized. This is where detailed measurements are of value and prove or disprove the early suppression of trees of the younger age classes by mistletoe.

In order to demonstrate the suppression in young trees, a series of measurements of the height growth of western yellow pine (*Pinus ponderosa*) were made in Spokane County, Washington. Two representative plots of 1 acre each, consisting of infected and uninfected reproduction and representing all age classes, were selected. The site was level bench land with a sparse stand of merchantable-sized trees. Some of these trees were severely infected with mistletoe [*Razoumofskya campylopoda*

[1] Weir, J. R. Larch mistletoe: some economic considerations of its injurious effects. U. S. Dept. Agr. Bul. 317, 25 p., 13 fig. 1916.

——— Mistletoe injury to conifers in the northwest. U. S. Dept. Agr. Bul. 360, 39 p., 27 fig. 1916. Literature cited, p. 39.

——— Some suggestions on the control of mistletoe in the national forests of the northwest. *In* Forestry Quart., v. 14, no. 4, p. 567-577. 1916.

[2] The term "suppression" is not used here in the ordinary forestry sense, but refers to a retardation of growth induced by parasitic organisms.

(Engelm.) Piper] and were the source of infection of the reproduction. The young trees were well distributed over the area and were dominant.

The results of this study are summarized in Table I and demonstrate very clearly the damage which may result from attack by this parasite on yellow pine.

TABLE I.—*Effect of mistletoe on yellow pine*

Age of tree.	Basis No.		Average height of trees.		Difference in height between infected and uninfected trees.
	Infected.	Uninfected.	Infected.	Uninfected.	
Years.			*Cm.*	*Cm.*	*Cm.*
4	8	50	15.22	25.38	10.15
5	10	27	22.84	35.53	12.69
6	14	42	35.53	50.76	15.22
7	9	35	48.22	71.06	22.84
8	19	20	53.29	78.68	25.38
9	10	23	63.45	93.90	30.45
10	7	16	73.60	109.13	35.53
Total	77	213	44.72	57.87	13.15

During the above study it was plainly observed that the length of the internodes of the infected trees, also the length of the terminal and lateral buds of the main shoot, were much shorter than those of the uninfected, and led to the following study on Douglas fir (*Pseudotsuga taxifolia*) in the Missoula River region near Missoula, Mont. The trees were infected with *Razoumofskya douglasii* Engelm. For this study trees were selected over an area without recourse to sample plots. The only point adhered to was the selection of trees on same type of site, condition of growth, and an average age of 18 years. The results are given in Table II

TABLE II.—*Effect of mistletoe on Douglas fir*

Trees seriously infected.										Trees vigorous, not infected.									
Basis (number of trees).	Average height growth of last four internodes.				Average dimension terminal bud.		Average dimension and number of lateral terminal buds.			Basis (number of trees).	Average height growth of last four internodes.				Average dimension terminal bud.		Average dimension and number of lateral terminal buds.		
	1	2	3	4	Length.	Breadth.	No.	Length.	Breadth.		1	2	3	4	Length.	Breadth.	No.	Length.	Breadth.
	Cm.	*Cm.*	*Cm.*	*Cm.*	*Mm.*	*Mm.*		*Mm.*	*Mm.*		*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Mm.*	*Mm.*		*Mm.*	*Mm.*
50	28	23	23	18	8	3	108	5	3	50	36	33	36	35	12	4	157	7	3 +

The foregoing results clearly demonstrate the effect of the formation of brooms and burls on the storage of food materials in the terminal buds and shoots. It is a well-known fact that in the terminal bud are stored

the elaborated food materials for its early development the following season. If this food material is reduced in amount by its becoming localized in other parts of the tree, the growth of the main shoot must be necessarily retarded (Pl. 37, B), and the bud itself will form earlier in the season and be much reduced in size. Two yellow-pine trees, each 8 years of age, one with a conspicuous infection with broom formation, the other entirely free from infection, were carefully observed to determine this point. The former not only started the elongation of the main shoot nine days later than the other tree, but ceased to develop altogether at the end of the first month. The shoot of the uninfected tree continued to elongate for two months and showed a gain of 11 inches over that of the infected tree. The trees grew under exactly similar conditions, and their root systems were practically equal in extent. Kirkwood [3] states that—

> It is improbable that the whole growth of the new leader is at the expense of the food stored in the bud alone. That from other parts also doubtless contributes, but the tendency is to crowd the formative materials toward the extrmities of the main shoot and the branch. In the sharing of these materials the main shoot leads and the branches follow in order of their importance. The principal growth, however, is undoubtedly at the expense of the locally stored materials, the substances stored elsewhere having their part to play in the development of the tissues in their immediate proximity.

The storage of food in the shoot and branches is exactly what the formation of brooms and burls prevents in a large measure in all parts of the tree above the seat of infection, and eventually results in retardation and the appearance of spike top or staghead. The writer has repeatedly called attention to this in previous publications. In order to demonstrate that there is an actual storage of food materials in mistletoe brooms greater than that of normal branches the results of an experiment may be given. Late in the month of October, 1914, after the leaves had fallen, 10 mistletoe brooms and 10 uninfected branches from points ranging from 5 to 15 feet above the former were cut from a western larch and thrown in a damp, shady ravine. In June of the following year the brooms and branches were examined with the following result: Practically all foliar spurs of the brooms developed needles in proportion of about one-third of the normal length. A few of the foliar spurs of the uninfected branches produced needles barely protruding from the bud scales, and in most cases there was no leaf production whatever. Observation of brooms in the crowns of larch cut late in the fall during logging operations showed in the production of needles in the spring that there must be a great localization of elaborated food materials in the branches of the brooms over that of the normal branches.[4] The latter showed no signs of foliation.

[3] KIRKWOOD, J. E. THE INFLUENCE OF PRECEEDING SEASONS ON THE GROWTH OF YELLOW PINE. *In* Torreya, v. 14, no. 7, p. 118. 1914.

[4] Certain parasitic fungi also cause a flow of building materials to the place of infection. See GOEBEL, K. E. EINLEITUNG IN DIE EXPERIMENTELLE MORPHOLOGIE DER PFLANZEN. p. 75. Leipzig und Berlin, 1908.

From the foregoing studies it may be concluded that the false mistletoes are serious agents in the suppression of young forest growth. Moreover, young growth once infected on the main stem (and it is usually so infected) can not recover and produce merchantable material. Suppression in young yellow pine even up to the sapling stage is of serious consequence. Very seldom do such trees ever overcome the early influence. This is also noticeable in trees of dense stands or when overtopped by older classes. The ultimate effect on the growth of the tree is exactly equivalent to lack of light, only worse. In the former case the excurrent growth is arrested, and the tree either develops into one continuous broom or dies; in the latter, a sapling of some merchantable value may result. It is clear that every effort should be made in regions of heavy mistletoe infection ro reduce the infection of reproduction by cutting all infected overtopping trees. Care should be taken to prevent the introduction of mistletoe-infected transplants in regions where the parasites do not occur.[5] Such regions, for example, are the Black Hills of South Dakota and several of the southeastern Montana forests.

SUMMARY

The height growth of young trees is greatly retarded by mistletoe. The effects to be observed are reduction in the length of the internodes, small dimensions of the terminal buds, and reduction in number. This result is caused by the localization of food materials at the seat of infection.

To reduce the chances of infecting young growth, all overtopping infected trees should be killed. Infected trees of any age should be killed if possible. Care should be taken that infected trees are not planted in regions where mistletoe does not occur.

PLATE 37

A.—*Pseudotsuga taxifolia* infected with *Razoumofskya douglasii*.

B.—Effect of an inoculation with *Razoumofskya campylopoda* on the height growth of 6-year-old *Pinus jeffreyi*. Culture made in a greenhouse at Missoula, Mont.

[5] Weir, J. R. Mistletoe injury to conifers in the northwest. U. S. Dept. Agr. Bul. 360, 39 p. 27 fig. 1916. Literature cited, p. 39.

DETERMINATION OF FATTY ACIDS IN BUTTER FAT: I[1]

By E. B. Holland, *Associate Chemist*, and J. P. Buckley, Jr., *Assistant Chemist, Massachusetts Agricultural Experiment Station*

INTRODUCTION

Since reporting a method (*14*)[2] for the determination of stearic acid in butter fat, work has been continued with a view of evolving a process for determining some of the remaining fatty acids. The object of the investigation was to deduce practical analytical methods which might serve to measure the effect of feed upon the composition of the resulting butter fat. Such methods, however, if quantitative and reasonably workable, would be applicable to other fixed oils and fats and play a part in the so-called technical examination of such products.

There are several distinct lines of procedure upon which methods for determining different fatty acids in a mixture might be based:

(1) Crystallization of the acids;

(2) Solubility of various salts;

(3) Fractionation of the acids *in vacuo;*

(4) Fractionation of methyl or ethyl esters.

The iodin absorption and the acetyl substitution are measures of unsaturated and of hydroxy acids, respectively. They are valuable adjuncts, but of limited rather than of general application.

Attention has been called in several instances to some of the inherent faults (*13, 14*) of the different schemes of separation, and a discussion of their relative merits appears unnecessary at this time. It will suffice to summarize the present application and limitation of the schemes.

Crystallization methods have been employed for the quantitative separation of stearic and of arachic (*27*) acids but seldom for other acids.

The lead-salt-ether method, or Gusserow (*7*)-Varrentrapp (*29*) process, for the separation of liquid from solid acids is the most prominent illustration of the salt-soluble methods which, as a rule, have not proved sufficiently discriminative for quantitative use.

Fractionation of the acids *in vacuo* has failed as an analytical process.

Fractionation of ethyl esters appeared applicable to many fatty acids, although chemists generally consider the process as having little quantitative significance. The experience gained in purifying fatty acids indicated that the method was practicable and at least worthy of additional study.

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[1] From the Department of Chemistry, Massachusetts Agricultural Experiment Station. Printed with the permission of the Director of the Station.

[2] Reference is made by number (italic) to "Literature cited," pp. 731-732.

EARLIER INVESTIGATIONS

At the present writing esterification methods have so wide an application and contributors to the literature on the subject are so numerous that only references bearing directly on the analysis of oils and fats will be cited.

Rochleder (*28*) studied the action of absolute alcohol and dry hydrochloric acid gas upon castor oil in the separation of glycerol.

Berthelot (*2, p. 311–312*) applied the reaction to a number of glycerides and showed that esters of the fatty acids were formed in addition to glycerol.

Juillard (*15, p. 239*) prepared methyl and ethyl esters of dihydroxystearic acid by boiling the acid with 10 times its weight of alcohol and 2 or 3 drops of sulphuric acid.

Fischer and Speier (*5*) esterified various organic acids with different amounts of absolute ethyl alcohol and dry hydrochloric or concentrated sulphuric acid, also of methyl alcohol and hydrochloric acid and noted the yield. They did not deduce a general method.

Haller (*8–11*) heated a variety of oils and fats with twice their weight of absolute methyl alcohol containing 1 to 2 per cent of hydrochloric acid, removed the glycerol and excess alcohol with water, or preferably brine, and fractionated the esters up to 194° C. (methyl caprylate) at atmospheric pressure and the residual esters *in vacuo*. The methyl oleate present in the myristic and higher fractions was removed by chilling and absorption on porous plates. When complete alcoholysis was not obtained, he recommended a second treatment with a new portion of acidulated alcohol, the employment of a larger amount of alcohol at the outset, or the addition of an inert solvent such as ether to facilitate the reaction, particularly in the case of butter fat and of drying oils which readily oxidize and polymerize. The production of a small amount of aldehyde was noted in some instances. Haller neutralized the esters with barium carbonate or a solution of sodium carbonate and dried over calcium chloride or anhydrous sodium sulphate.

Phelps and Hubbard (*18*) esterified succinic acid with ethyl alcohol and hydrochloric acid, and secured the greatest yield with maximum dehydration. In other experiments (*19*) the addition of anhydrous zinc chlorid enhanced the reaction.

Complete esterification of 50 gm. of benzoic acid (*26*) was obtained by treating for 4 hours with 400 c. c. of absolute alcohol containing 1.25 per cent of dry hydrochloric acid and 10 gm. of anhydrous zinc chlorid, or for 3 hours with 200 c. c. of absolute alcohol and 2 gm. of sulphuric acid.

Several other chlorids (*24, p. 296–297; 20–25*) proved nearly as efficient as zinc chlorid under like conditions of operation.

Meyer (*17*) employed substantially the Haller method with cottonseed oil, but increased the amount of methyl alcohol to four times the weight of the oil, and obtained a yield of about 90 per cent.

Elsdon (*3, 4*) employed the Haller method with coconut oil and palm-kernel oil, fractionated the resulting methyl esters *in vacuo*, and refractionated to constant boiling point. He stated that the process had qualitative and a considerable amount of quantitative value, but was too lengthy for ordinary use.

Kailan (*16*) found that ether, benzene, and carbon tetrachlorid did not accelerate the esterification of benzoic acid with absolute alcohol and hydrochloric acid or with dilute alcohol and acid.

Wolff and Scholze (*30*) used a dilute sodium-bicarbonate solution to purify the esters when shaking out with ether.

Abderhalden and Kautzsch (*1*) esterified the silver salt of an amino acid by boiling with an excess of ethyl iodide.

Grandmougin, Havas, and Guyot (*6*) showed how an organic acid after treatment with sodium methylate might be converted by means of an excess of dimethyl sulphate into the methyl ester of the organic acid and sodium methyl sulphate.

Possibly methyl sulfonic acid might be substituted in some instances for dimethyl sulphate or methyl halide as indicated by an English patent, No. 9359.[1]

Hauser and Klotz (*12*) esterified organic acids with alcohols by passing the vapors over glucinum oxide heated to 310° C.

Permissible space does not allow one to do justice to the articles cited.

PRELIMINARY WORK

Further study of esterification was undertaken with a view of securing a method for determining the percentage of another insoluble acid besides stearic and oleic in butter fat. Lauric acid was naturally the most promising, on account of the lower boiling point of its esters, although myristic acid was also considered a possibility. The percentage of lauric acid, together with ordinary analytical data and the amount of stearic acid determined by crystallization, would permit a satisfactory calculation of the remaining insoluble acids.

MATERIAL.—At the outset the insoluble acids were employed for esterification with an idea that the previous elimination of soluble acids and of glycerol would be an advantage. In reality such did not prove the case, as water, a limiting factor in esterification, was produced in the reaction between fatty acids and alcohols, while glycerol was produced in the case of fats (glyceryl esters) and alcohols as shown by the following equations:

$$\underset{\text{fatty acid}}{RCOOH} + \underset{\text{alcohol}}{R_1OH} + \text{catalyzer} = \underset{\text{ester}}{RCOOR_1} + \underset{\text{water}}{H_2O}$$

$$\underset{\text{fat}}{(RCOO)_3C_3H_5} + \underset{\text{alcohol}}{3R_1OH} + \text{catalyzer} = \underset{\text{ester}}{3RCOOR_1} + \underset{\text{glycerol}}{C_3H_5(OH)_3}$$

[1] Chem. Abs., v. 6, no. 4, p. 535. 1912.

Furthermore, the employment of the original product was preferable from an analytical standpoint to say nothing of the time and labor

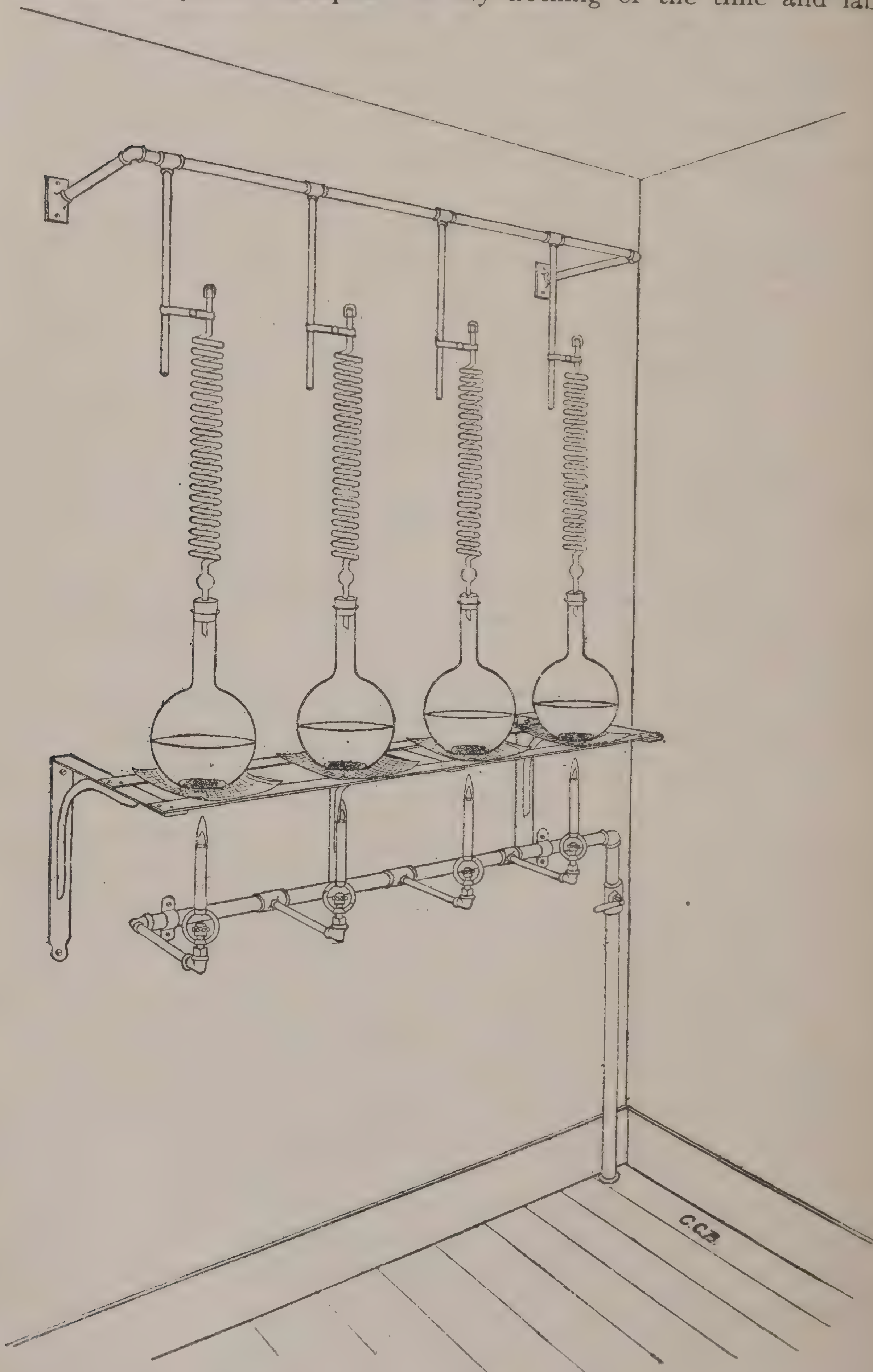

FIG. 1.—Apparatus employed in esterification.

involved in the preparation of a large stock of insoluble acids. Butter fat was used in all subsequent work.

ESTERIFICATION (fig. 1).—Earlier investigators have indicated various methods of esterification. Absolute alcohol with a mineral acid catalyzer appeared the most practicable for analytical purposes and was adopted tentatively. Between methyl and ethyl alcohols there was little choice, except as to cost and convenience, although the methyl esters have a somewhat lower boiling point. Ethyl alcohol was used exclusively.

The preparation of dry ethyl alcohol substantially free from aldehydes was found considerable of a problem. For dehydration neither metallic calcium nor any quicklime from the usual sources proved efficient. A granulated caustic lime containing about 95 per cent of calcium oxid was eventually obtained from the manufacturers which would produce a dry alcohol on the third distillation.

Different schemes have been suggested for the removal of aldehydes, such as oxidation with silver nitrate, potassium permanganate, or potassium bichromate, polymerization with caustic alkali or fractional distillation, and all were tried in some form or other. The following process finally proved satisfactory and was adopted:

Approximately 2 liters of alcohol were fractionated in a water bath over 600 to 700 gm. of caustic lime and 2 to 3 gm. of caustic soda. The main portion of the distillate, the first and last being rejected, was refractionated twice in a similar manner over fresh lime and soda. The rejected portions were united and retreated.

Dry hydrochloric acid or concentrated sulphuric acid has been the catalyzer almost invariably employed by different workers for esterification. The former with a greater hydrogen-ion concentration is apparently less efficient, gram for gram, than the latter, and is generally used in larger amounts. This may be due in part to loss of hydrochloric acid as ethyl chlorid by volatilization, but more likely to the dehydrating effect of sulphuric acid. By using a larger quantity of alcohol, together with certain chlorids, the yield of esters with hydrochloric acid may be increased, as shown by Phelps.

A considerable excess of alcohol is also required to insure the necessary mass action irrespective of its dehydrating action. For esterifying 150 gm. of butter fat, 400 c. c. of absolute ethyl alcohol containing 8 gm. of dry hydrochloric acid or 4 c. c. of concentrated sulphuric acid were employed. This amount of alcohol furnished about 11 times that needed for combination with the fatty acids.

The use of a neutral solvent, such as ether, did not appear to accelerate esterification and was omitted after a few trials. Butter fat diffused rapidly through the acid alcohol on boiling, and the solution generally cleared in a few minutes. Short boiling periods were tried, but 24 hours were considered more reliable and adopted.

PURIFICATION OF ESTERS.—After completing the esterification and cooling the solution, the esters must be precipitated and freed from mineral acid, glycerol, and excess alcohol.

Attention has already been called to various methods of neutralizing, purifying, and drying the esters, as described by other workers. Most of these processes and innumerable modifications were given careful trial, but the readiness with which at least a portion of the butter-fat esters hydrolyzed precluded the use of water in their purification. This was an extremely exacting condition and called for a salt soluble in alcohol, neutral in reaction, and with dehydrating properties. Dry magnesium

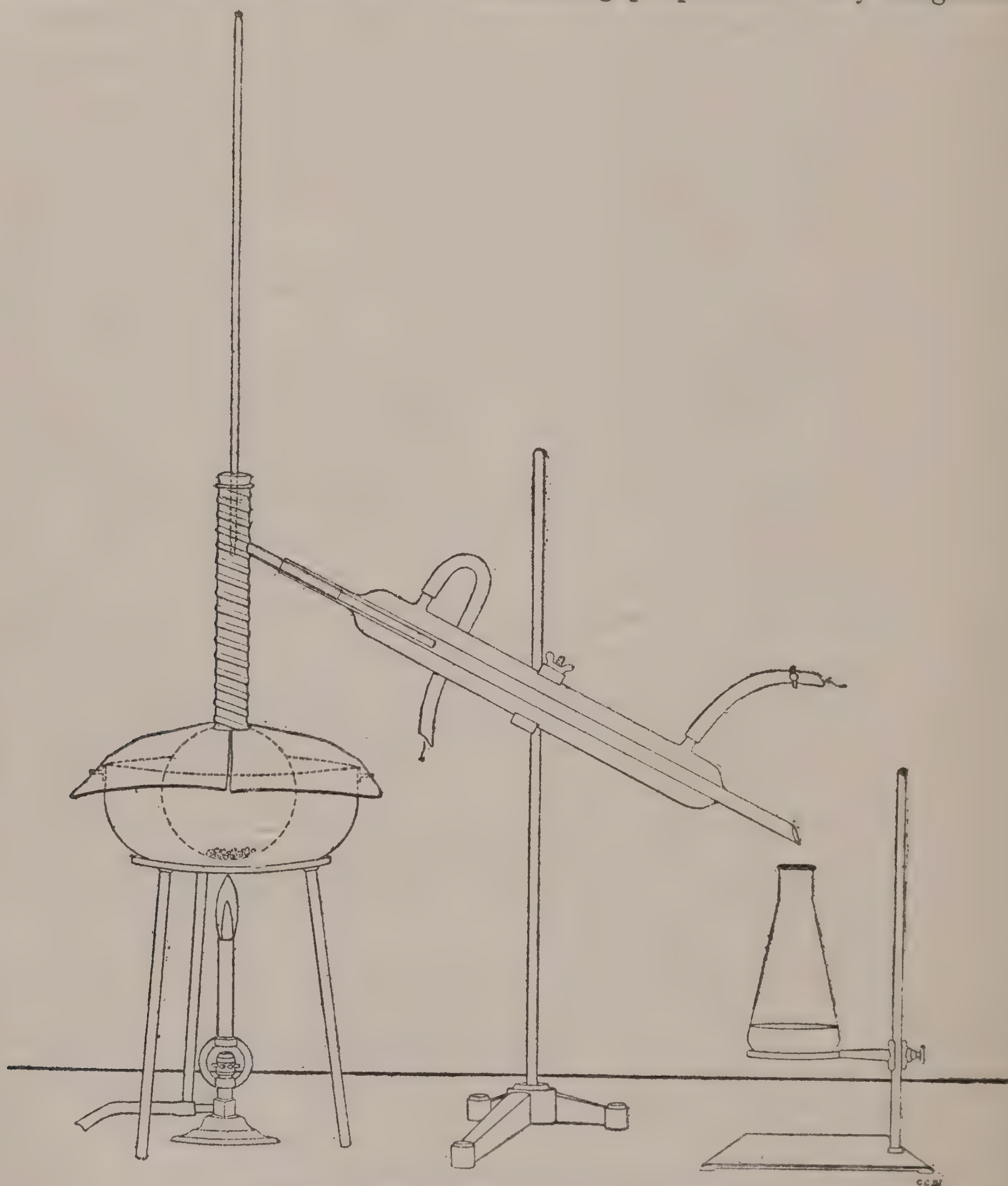

FIG. 2.—Apparatus employed in fractionation.

chlorid satisfactorily met these requirements, and furthermore was cheap and easily procured. On the addition of the dry salt in the presence of ether, a rapid separation of esters was obtained. The underlying solution was drawn off by means of a separatory funnel, and the esters were purified by "shaking out" several times with ether and a saturated alcoholic solution of magnesium chlorid. The ether facilitates the separation and should always be added before the magnesium chlorid.

All the agitation necessary can be obtained by reversing the separatory funnel several times, which gives a clear separation much quicker than a more violent shaking. The resulting esters contain ether and probably some alcohol, but do not require neutralizing or drying.

FRACTIONATION (fig. 2).—Fractionation of the esters *in vacuo* by means of a Bruehl or other type apparatus was found impracticable as a quantitative process for the reason that a constant level of the liquid in the distilling flask and a constant pressure were prime requisites for a definite fraction, neither of which could be successfully maintained with the facilities at hand. Furthermore, the use of such apparatus necessitates a certain aptitude or technic not possessed by all analysts.

Fractionation at atmospheric pressure required a high temperature, but proved feasible. Gas could not be used as a direct source of heat owing to fluctuations in pressure, influence of air currents, tendency to decompose the esters, etc., but when applied to a bath of superheated valve oil proved entirely satisfactory. This oil is a cheap commercial product that will safely withstand a temperature of over 400° C. when covered and may be used three times, possibly more, without appreciable loss of efficiency. All the exposed surface of the side-neck flask should be wound with asbestos paper to prevent chilling the vapors and breakage of the flask.

For some reason fractionation of the purified esters proved impracticable, possibly due to the influence of a relatively large amount of the higher esters particularly oleic, but was readily accomplished after a preliminary distillation. Accurate results are dependent in large measure on a slow, steady rise in temperature during the first distillation and subsequent fractionation. Glass beads were found helpful in boiling.

TABLE I.—*Boiling point and range of fractions (uncorrected) of esters of butter fat*

Ester.	Boiling point.	No.	Esters.	Range of fractions.
	°C.			°C.
Ethyl butyrate	119.9–121	1	Ethyl butyrate, caproate, and oleate	125–180
Ethyl caproate	165–167			
Ethyl caprylate	205–208	2	Ethyl caproate, caprylate, and oleate	180–225
Ethyl caprate	243–245			
Ethyl laurate	269	3	Ethyl caprylate, caprate, and oleate	225–270
Ethyl myristate	295			
Ethyl palmitate	?	4	Ethyl caprate, laurate, and oleate	270–300
Ethyl stearate	?			
Ethyl oleate	?	5	Ethyl laurate, myristate, and oleate	300–325
		6	Ethyl myristate, palmitate, and oleate	325–365

The range of the several fractions is more or less arbitrary, being dependent on the speed of distillation and the distance the vapors have to rise. The object was to secure fractions that did not contain more than two esters in addition to oleic ester, and, furthermore, adjacent fractions should contain approximately one-half of the saturated ester appearing

in each. The reported boiling point of the ethyl ester of a number of fatty acids that occur in butter fat, together with the range of the several fractions as determined by analysis for a "high" side-tube 500-c. c. distillation flask, may be noted in Table I.

The apparent lack of agreement is probably due to the greater distance the vapors have to rise in practical fractionation and to the influence of a gradually increasing amount of ethyl oleate in the several fractions. Analysis seemed to be the only method for accurately establishing the required range. Hempel tubes or similar apparatus could not be employed to break up the distillate.

As the preliminary work advanced, the results became more concordant and indicated that it was possible to determine not only lauric and myristic acids but also caproic, caprylic, and capric acids. A part of the butyric acid was recovered, but the main portion was evidently lost during the purification of the esters or distilled over with the ether, owing to the greater solubility and volatility of this ester.

Analysis of fractions.—Only the determinations of saponification and of iodin numbers by the usual methods employed with oils and fats were required.

Calculation of results.—Having determined the weight, saponification and iodin numbers of the several fractions, the analyst must ascertain whether the range of the fractions had been accurately established. If correctly fractionated, the percentage of the different ethyl esters in the several fractions can be calculated algebraically and their weight computed, from which the percentage of the corresponding acids in the butter fat can be determined.

As a matter of convenience, the data necessary for these calculations have been compiled in Table II.

Table II.—*Fatty acids and their ethyl esters*

[C, 12.005; H, 1.008; O, 16.000; K, 39.10; I, 126.92]

Acid.	Molecular weight of acid.	Molecular weight of ester.	Saponification No. of ester.	Iodin No. of ester.	Reciprocal.	Conversion factor, ester to acid.
			Mgm.			
Acetic	60.042	88.084	636.983			0.68164
Butyric	88.084	116.126	483.165			.75852
Valeric	102.105	130.147	431.113			.78454
Caproic	116.126	144.168	389.185			.80549
Caprylic	144.168	172.210	325.812			.83716
Capric	172.210	200.252	280.187			.85997
Lauric	200.252	228.294	245.771			.87717
Myristic	228.294	256.336	218.885			.89060
Palmitic	256.336	284.378	197.301			.90139
Stearic	284.378	312.420	179.592			.91024
Arachic	312.420	340.462	164.800			.91764
Oleic	282.362	310.404	180.758	0.81777	1.22284	.90966
Erucic	338.446	366.488	153.096	.69263	1.44377	.92348
Linolic	280.346	308.388	181.940	1.64624	.60744	.90907
Linolenic	278.330	306.372	183.137	2.48561	.40232	.90847
Clupanodonic	276.314	304.356	184.350	3.33609	.29975	.90786
Ricinoleic	298.362	326.404	171.897	.77769	1.28586	.91409
Dihydroxystearic	316.378	344.420	162.906			.91858

As an illustration of the method of calculation, take the fifth fraction of a sample of butter fat (Table III) which weighed 50.69 gm., had a saponification number of 219.280 and an iodin number of 14.055, and was intended to contain ethyl laurate, myristate and oleate.

The iodin number, convertible to ethyl oleate by the factor 1.22284, is equivalent to 17.187 per cent.

Then $1.00-0.17187$, or 0.82813, is the percentage of the residual esters in the fraction and $219.280-(0.17187 \times 180.758)$, or 188.213, their alkali-consuming power. The latter divided by the former gives a saponification number of 227, a figure between that of ethyl laurate (245.771) and that of ethyl myristate (218.885), as anticipated.

Let x indicate ethyl laurate and y ethyl myristate, then

$$x+y=0.82813$$
$$245.771x+218.885y=188.213$$
$$x=25.842 \text{ per cent of ethyl laurate}$$
$$y=56.971 \text{ per cent of ethyl myristate.}$$

The percentages 0.25842, 0.56971, and 0.17187 multiplied by the weight of the fraction (50.69 gm.), gives the weight of the several esters in the fraction 13.099, 28.879, and 8.712, which by their respective factors 0.87717, 0.89060, and 0.90966 may be converted into the weight of the several fatty acids in the fraction. The total weight of a fatty acid divided by the weight of fat taken (300) gives the percentage of that acid in the butter fat. The same method of calculation is followed in each of the other fractions.

METHOD IN DETAIL

REAGENTS.—Alcohol: absolute.

Hydrochloric acid: dry, generated by dropping concentrated sulphuric acid into a mixture of concentrated hydrochloric acid and sodium chlorid and dried by passing through concentrated sulphuric acid.

Sulphuric acid: concentrated, heated to 225° C.

Ethyl ether: anhydrous, freshly distilled over metalic sodium.

Magnesium chlorid: dry powder, neutral. Dried at 75° to 80° C.

Magnesium chlorid solution: 250 gm. of dry powder to 500 c. c. of absolute alcohol.

ESTERIFICATION AND PURIFICATION OF ESTERS.—Into a 1,000-c. c. flat-bottom globe flask are brought 150 gm. of filtered fat, together with 400 c. c. of alcohol previously charged with 8 gm. of dry hydrochloric acid, or 4 c. c. of concentrated sulphuric acid and a number (25) of glass beads. The flask is connected with a long spiral or other form of reflux condenser, and the mixture carefully boiled on 30-mesh Nichrome wire gauze for 24 hours.

After esterification the contents of the flask are cooled, 50 c. c. of ether and 150 gm. of magnesium chloride added, rotated to hasten saturation, transferred to a 1,000-c. c. pear-shaped separatory funnel and allowed to stand until a clear separation is secured. The underlying layer is drawn off into the original flask and the esters carefully shaken out two or three times with 25 to 50 c. c. of ether and 50 c. c. of an alcoholic solution of magnesium chlorid. Violent shaking causes a slow separation. After the removal of the final washing, the clear, purified esters are filtered through a firm, close-textured paper into a 500-c. c. round-bottom "low" side-tube distillation flask. The filter is extracted with ether which is run into the original globe flask containing the alcoholic layer and washings from the esters. More ether is added until a ready separation is obtained and the solution again transferred to the separatory funnel and allowed to stand several hours to recover any occluded esters which are then washed several times with ether and magnesium chlorid solution as previously described, and filtered into the distillation flask containing the first portion.

FRACTIONATION.—A number (50) of glass beads are placed in the side-neck distillation flask which is connected with a 12-inch Liebig condenser and heated in a bath of superheated valve oil for distillation of the esters. The exposed portion of the flask should be covered with asbestos paper and the condenser filled with cold water at the outset but no circulation should be permitted during the distillation. The temperature should be raised slowly. After the ether and alcohol are expelled, the entire distillate between 85° and 365° C. is collected and constitutes from 110 to 120 gm. with butter fat.

The distillate from two portions representing 300 gm. of butter fat and a number (50) of glass beads are brought into a 500-c. c. round-bottom "high" side-tube distillation flask which is connected with a Liebig condenser and heated as previously described. Particular care should be exercised in heating the oil bath so as to insure a slow, steady rise in temperature taking at least 80 minutes from the beginning of the first fraction to the completion of the last. The required range of every fraction must be accurately established with the apparatus employed by analysis (See Table I).

The volatility of the esters and the readiness with which they hydrolyze necessitates careful treatment of the fractions, which should be collected in tared flasks, weighed, and the saponification and iodin numbers determined as soon as possible.

APPLICATION OF THE METHOD

A sample of dry filtered butter fat churned from sweet cream from mixed milk of the Experiment Station herd was taken for examination. An analysis of the fat and of its insoluble acids gave the following results, which indicate a normal product.

FAT

Saponification number	mgm..	231.453
Acid number	mgm..	2.183
Ether number (e)	mgm..	229.270
Iodin number		27.999
Equivalent in oleic acid	per cent..	31.145
Total fatty acids ($1.00-0.00022594\ e$)	per cent..	94.819
Insoluble fatty acids (by alcoholic saponification)	per cent..	87.500
Soluble fatty acids (by difference)	per cent..	7.319
Glycerol ($0.00054703\ e$)	per cent..	12.542

INSOLUBLE ACIDS

Neutralization number	mgm..	221.890
Iodin number		28.125
Equivalent in oleic acid	per cent..	31.285
Stearic acid (by crystallization)	per cent..	13.010

The fat was esterified, with dry hydrochloric acid as a catalyzer, the esters purified and fractionated as described in the method.

In Table III are given the range, weight, saponification, and iodin numbers of the several fractions. From these data the percentage and weight of the different esters in the fractions were calculated (Table IV) and the weight of the corresponding acids and their percentage computed on the basis of the original fat (Table V).

TABLE III.—*Weight and analysis of fractions*

No.	Range of fraction.	Weight of fraction.	Saponification No.	Iodin No.	Ethyl oleate.
	°C.	Gm.	Mgm.		Per cent.
1	125–180	5.0835	417.209	4.753	5.812
2	180–225	4.4400	346.396	8.091	9.894
3	225–270	6.8855	279.111	11.471	14.027
4	270–300	15.2990	240.983	12.846	15.709
5	300–325	50.6900	219.280	14.055	17.187
6	325–365	84.8800	205.955	16.691	20.410

TABLE IV.—*Percentage and weight of esters recovered*

No.	Ethyl butyrate.		Ethyl caproate.		Ethyl caprylate.		Ethyl caprate.		Ethyl laurate.		Ethyl myristate.		Ethyl palmitate.		Ethyl oleate.	
	P. ct.	Gm.	P. ct.	Gm.	P. ct.	Gm.	P. ct.	Gm.	P. ct.	Gm.	P. ct.	Gm.	P. ct.	Gm.	P. ct.	Gm.
1	42.708	2.171	51.480	2.617	......	......	......	......	......	......	......	......	......	......	5.812	0.295
2	......	......	55.128	2.448	34.978	1.553	......	......	......	......	......	......	......	......	9.894	.439
3	......	......	......	......	28.210	1.942	57.763	3.977	......	......	......	......	......	......	14.027	.966
4	......	......	......	......	......	......	15.763	2.412	68.528	10.484	......	......	......	......	15.709	2.403
5	......	......	......	......	......	......	......	......	25.842	13.099	56.971	28.879	......	......	17.187	8.712
6	......	......	......	......	......	......	......	......	......	......	55.736	47.309	23.854	20.247	20.410	17.324
Total	......	2.171	......	5.065	......	3.495	......	6.389	......	23.583	......	76.188	......	20.247	......	30.139

TABLE V.—*Weight and percentage of fatty acids recovered*

Acid.	Gm.	Per cent.	Acid.	Gm.	Per cent.
Butyric acid (partial recovery)	1. 647	0. 549	Myristic acid	67. 853	22. 618
Caproic acid	4. 080	1. 360	Palmitic acid (partial recovery)	18. 250	6. 083
Caprylic acid	2. 926	. 975	Oleic acid (partial recovery)	27. 416	9. 139
Capric acid	5. 494	1. 831			
Lauric acid	20. 686	6. 895			

The recovery of ethyl butyrate was incomplete, owing to its high solubility and volatility; that of ethyl palmitate was due to inability to continue the distillation, owing to insufficient volume of higher esters and excessive heat requirements. Ethyl oleate appeared in all fractions, gradually increasing with the temperature.

The percentage of the different fatty acids in the butter fat are presented in Table VI. Butyric acid of the soluble acids and palmitic acid of the insoluble were determined by difference, stearic acid by crystallization, as previously stated, and oleic acid from the iodin number of the insoluble acids. No allowance was made for unsaponifiable matter in the calculation.

The alkali-consuming and glycerol-combining powers of the several acids are also recorded and confirm the results secured in large measure. The glycerol requirements of the constituent acids slightly exceed that of the fat, for the reason that no allowance was made for the free fatty acids present.

TABLE VI.—*Percentage of fatty acids in butter fat*

Fatty acids.	Amount present.	Saponification number.	Glycerol.
SOLUBLE ACIDS:	*Per cent.*	*Mgm.*	*Per cent.*
Butyric acid	[a] 3. 153	20. 084	1. 099
Caproic acid	1. 360	6. 571	. 359
Caprylic acid	. 975	3. 795	. 208
Capric acid	1. 831	5. 966	. 326
Total		36. 416	1. 992
Calculated	7. 319	37. 299	
INSOLUBLE ACIDS:			
Lauric acid	6. 895	19. 319	1. 057
Myristic acid	22. 618	55. 588	3. 041
Palmitic acid	[a] 19. 229	42. 089	2. 302
Stearic acid	11. 384	22. 461	1. 229
Oleic acid	27. 374	54. 395	2. 976
Total		193. 852	10. 605
Determined	87. 500	194. 154	
SOLUBLE AND INSOLUBLE ACIDS:			
Total		230. 268	12. 597
Determined or calculated from fat	94. 819	231. 453	12. 542

[a] By difference.

In a later article attention will be called to additional analyses of butter fat and to the results reported by other workers employing different methods.

SUMMARY

The direct esterification of butter fat with subsequent fractionation of the resulting esters has proved an accurate and practical method for the determination of five of the fatty acids in butter.

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